BOTANICAL GAZETTE

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THE BOTANICAL GAZETTE

EDITORS:

JOHN MERLE COULTER AND CHARLES REID BARNES

VOLUME XLVIII

JULY–DECEMBER, 1909

WITH EIGHTEEN PLATES AND ONE HUNDRED AND TWENTY-ONE FIGURES

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ERRATA

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Pp. 289, 290, transpose figures 18 and 19. The legends, as now placed, will then be correct.

P. 373, footnote 30, last line, for 1295 read 85.

P. 489, Amitosis in Synchytrium, for 136 read 127.

P. 489, Anatomy of Microcycas calocoma, for 127 read 139.

P. 490, after Dorety, Helen A., insert 139.

P. 490, under contributors, Griggs, R. F., for 136 read 127.

P. 491, Griggs, Robert F., for 136 read 127.

P. 492, Heteroschizis in Synchytrium, for 140 read 131.

P. 493, Microcycas calocoma, vascular anatomy of, for 127 read 139.

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P. 76, footnote 23, for Newcombe, F. W.; read Newcombe, F. C.

P. 158, line 9, for Kränzlein read Kränzlin.

P. 237, footnote 6, for VON read VAN.

P. 228, line 8 from bottom, for Function read Functions.

P. 228, footnote 6, for Graves, A. B., read Graves, A. H.

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THE BOTANICAL GAZETTE

July 1909

Editors: JOHN M. COULTER and CHARLES R. BARNES

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VOLUME XLVIII NUMBER 1

BOTANICAL GAZETTE

JULY 1909

VARIATION OF FUNGI DUE TO ENVIRONMENT[1]

F. L. STEVENS AND J. G. HALL

(WITH THIRTY-SEVEN FIGURES)

The effects of environment, climatic condition, soil fertility, the presence of unusual chemicals, the water relation, and what not, upon the form and characters of seed plants, are well known to the plant physiologist, and have been the subject of numerous studies. These factors are even utilized by the practical man to bring about desired variation.

That fungi vary similarly will not be doubted by any who have had to do with fungi in artificial cultures. The kind and degree of such variation, we dare say, will be a surprise to any who have not made special study of this subject.

Our knowledge of the seed plants, owing to man's long acquaintance with them, their larger size, and comparative stability, is considerable; yet even with them the limiting of genera, species, varieties, etc., presents difficulty, if we may judge from the rich literature upon phanerogamic taxonomy. The fungi, because of their immense number of species, variety of forms, minuteness, paucity of distinguishing characters, complexity of life-history (mostly unknown), peculiar biologic host relations (almost entirely unknown), and because of man's short acquaintance with them and their unknown but apparently vast range of variability, present as yet baffling problems of relationship and classification.

The object of the present paper is to call attention to the kind and degree of environmental variation found in a few species of fungi that have been studied by the authors during the past four or five years,

[1] Read in part at the Baltimore meeting of the Botanical Society of America, December, 1908.

and in some instances to analyze the causes of these variations, to the end that the factor of environmental variation may be more clearly recognized as a problem of mycological taxonomy.

We shall consider these variations under the causes that produce them.

I. Density of colonies

SEPTORIA PETROSELINI DESM. VAR. APII BR. & CAV., FROM CELERY

This fungus, when plated so that the spores lay thinly scattered, produced colonies which were ultimately black, 1 to 2^{mm} in diameter,

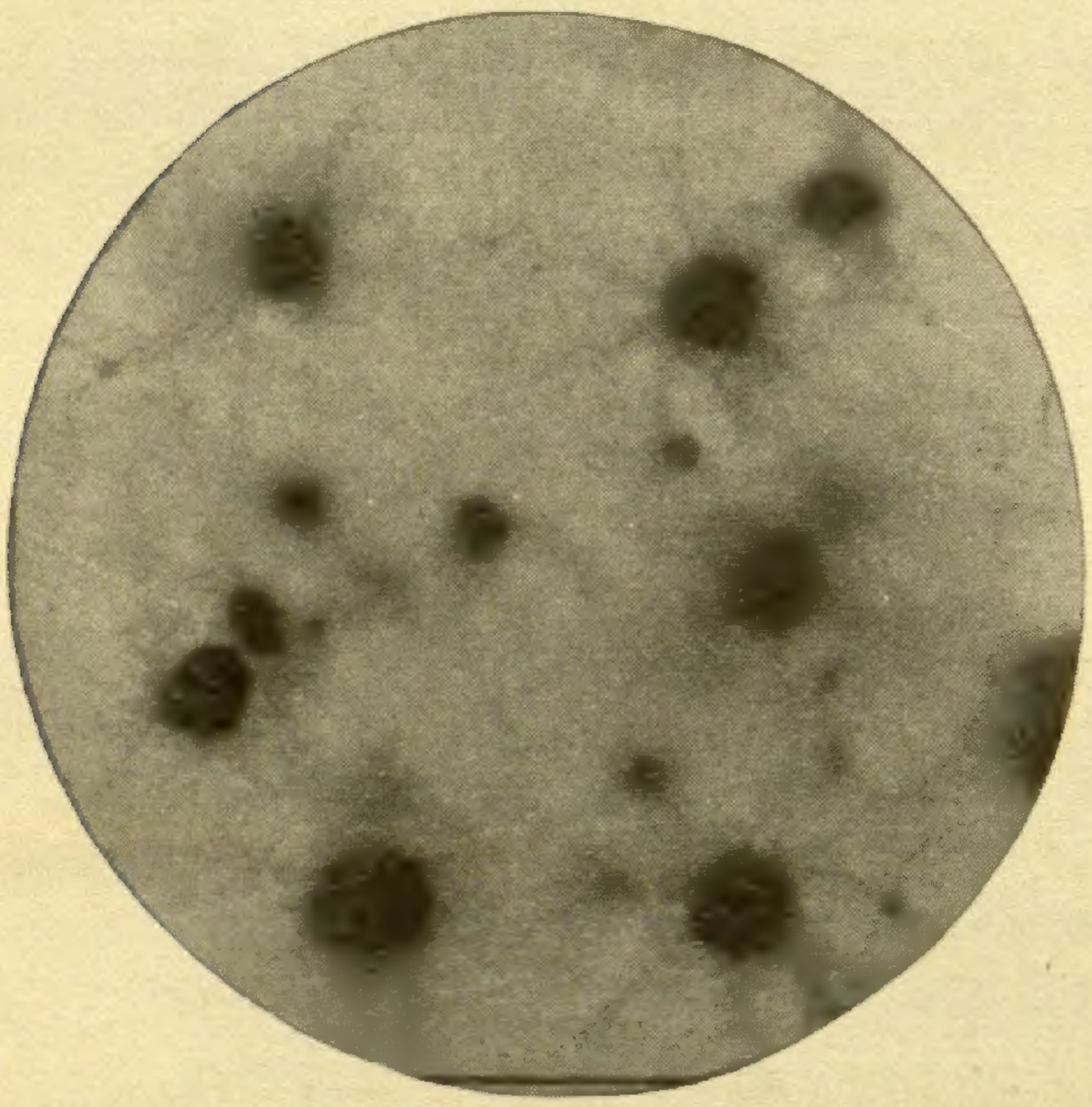

FIG. 1

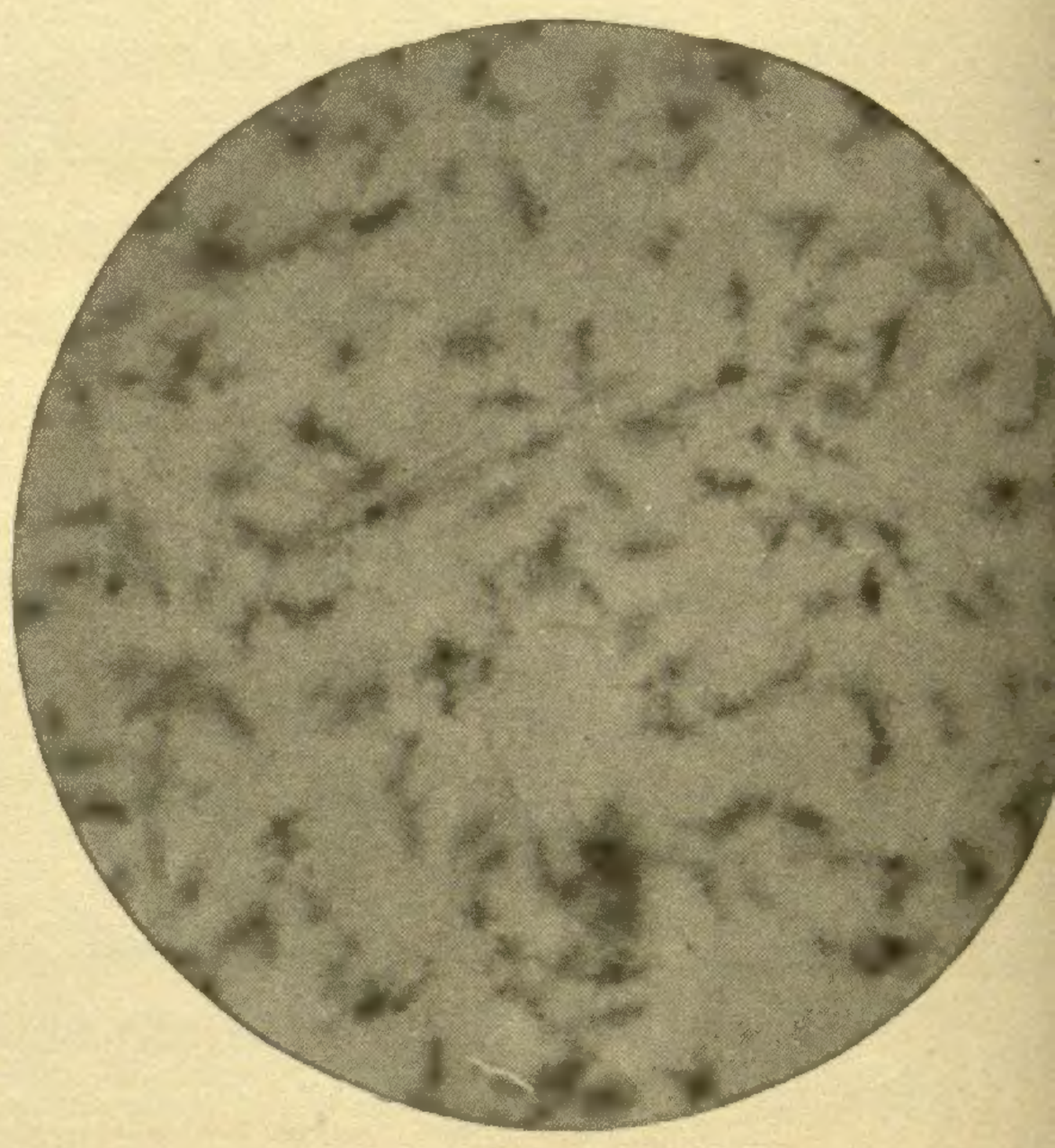

FIG. 2

FIG. 1. *Septoria Lycopersici* Speg., showing formation of normal pycnidia on portion of thinly sown plate culture.—FIG. 2. *Septoria Lycopersici* Speg., showing absence of pycnidia on thinly sown portion of plate culture; magnification same as in *fig. 1*.

with pycnidia of normal character. If plated so that the spores lay in large numbers per square centimeter, it produced colonies which reached a size of only about 0.5^{mm} and became ultimately black, containing ordinary pycnidia, bearing spores in the normal way. When plated so that there were still more spores per square centimeter, the colonies never became black and no pycnidia were produced; but on the contrary, multitudes of spores were borne uncovered, in clumps upon simple hyphae.

SEPTORIA LYCOPERSICI SPEG., FROM TOMATO

Spores from pure culture were plated in 4 per cent. pea agar in various dilutions.

One plate developed 5 to 6 colonies per square millimeter and each colony proceeded to normal pycnidial development. Another plate developed 21 to 23 colonies per square millimeter, and all proceeded to form naked conidia with no indication of pycnidia. Portions of these two plates are represented by photomicrographs (*figs. 1, 2*). Drawings of the naked spores showing the detail of their formation are given in *fig. 3*. Occasionally plates with as many as 30 colonies per square millimeter were found with both pycnidia and naked spores.

Pycnidia not visible at the fifth day may be well formed by the eighth day and extrude masses of pink spores about the twenty-first day. Occasionally pycnidia are well developed on the fourth day. When naked spores develop they normally appear a few days later than do pycnidia, e. g., a plate thinly sown January 12, 1907, gave many pycnidia on January 15; while a thickly sown plate, under conditions otherwise precisely parallel, did not give naked spores until January 22. This Septoria forms a typical determinate colony, i. e., even with unlimited room, it proceeds only to a certain size of development.

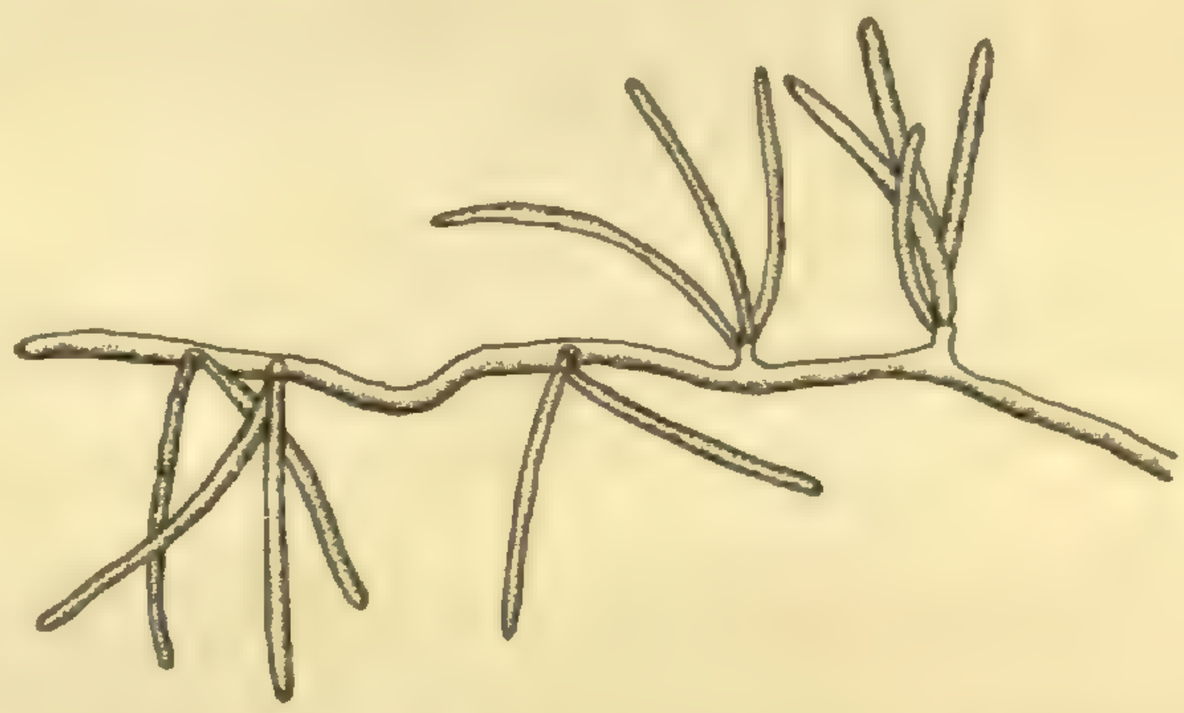

FIG. 3.—Mode of formation of naked spores under influence of crowded culture.

SEPTORIA CONSIMILIS E. & M., FROM LETTUCE

When sown thinly, colonies reached a size of 2 to 3^{mm} in diameter; when sown thickly, they became no more than 0.2^{mm} in diameter. There was no interference with color development or formation of pycnidia by thick sowing with this species.

With two of these septorias, thick plating, other conditions being the same, so changed their character that not only would the species be considered as different, but the fungus would be shifted from the order Sphaeropsidales to the order Hyphomycetales (Hyplaesa of SACCARDO).

A similar change of habit is well known in the genus Fusarium, which in culture, crowded or not, often abandons acervulus formation, thus changing its systematic position from the Tuberculariaceae to the Mucedinaceae. The genera Colletotrichum and Gloeosporium similarly abandon acervulus formation and thus suffer still greater taxonomic disturbance by moving from the Melanconiales to the Hyphomycetales.

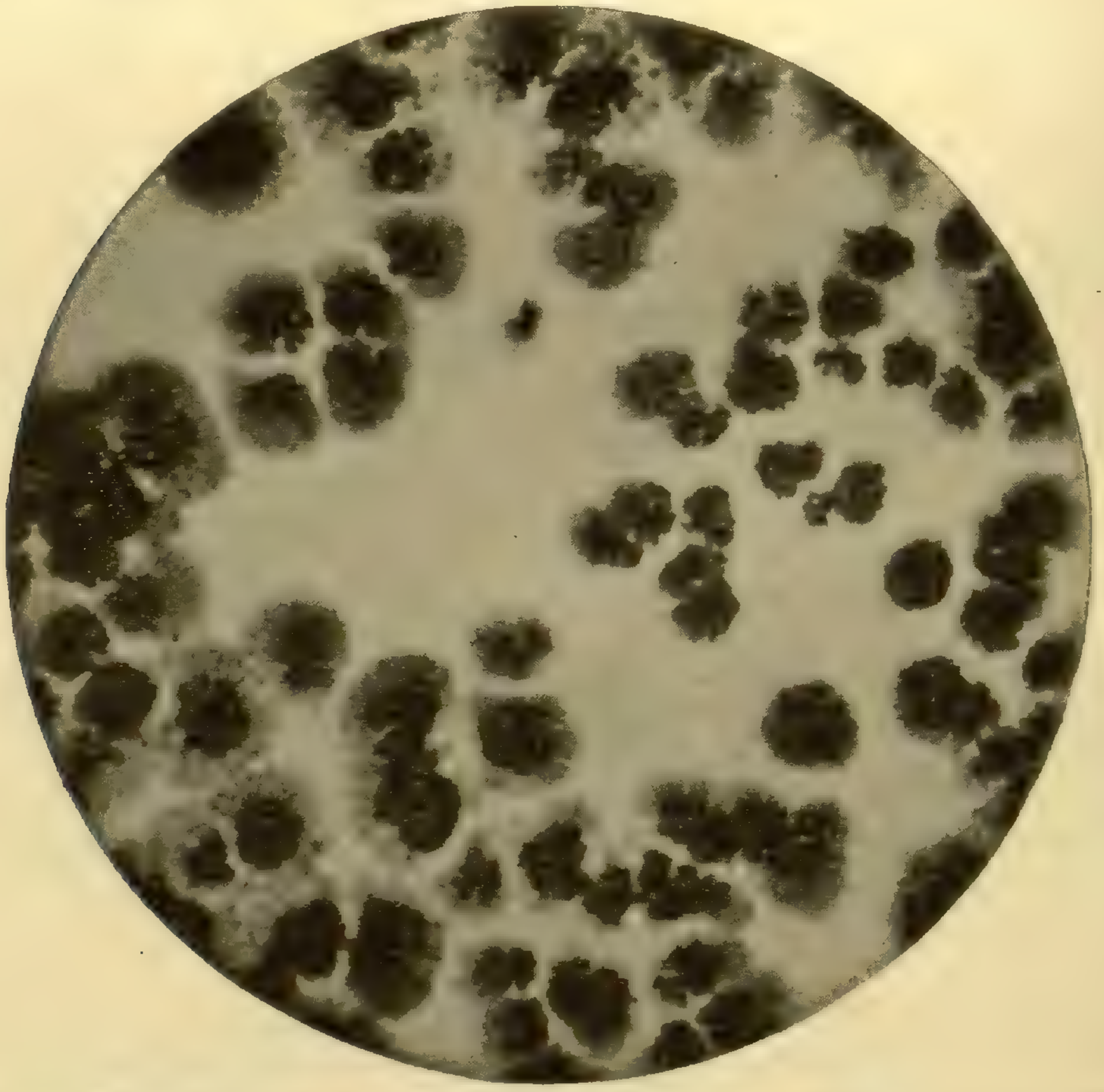

FIG. 4.—*Volutella fructi* S. & H., showing colonies on thinly sown plate culture.

ASCOCHYTA CHRYSANTHEMI STEVENS, FROM CHRYSANTHEMUM

This fungus was plated January 12, 1907. Myriads of pycnidia were present four days later. Thick plating caused no inhibition of pycnidial formation, no naked spores, and no constant effect upon the number of pycnidia produced.

VOLUTELLA FRUCTI S. & H., FROM APPLE

Thinly sown, the colonies were large, of indeterminate growth, showing dark centers with pale borders (*fig. 4*). Thickly sown, growth was inhibited and these characters lost (*fig. 5*).

SPERMOEDIA PASPALI FRIES, FROM PASPALUM[2]

Spores of this fungus were sown January 19, 1907, in plates giving colony densities of 90, 54, 30, 14, and 1 per square millimeter.

At all of these densities germination was practically 100 per cent. and growth proceeded equally in all plates during the early stages. On February 11 it was noted that all colonies which came nearly in

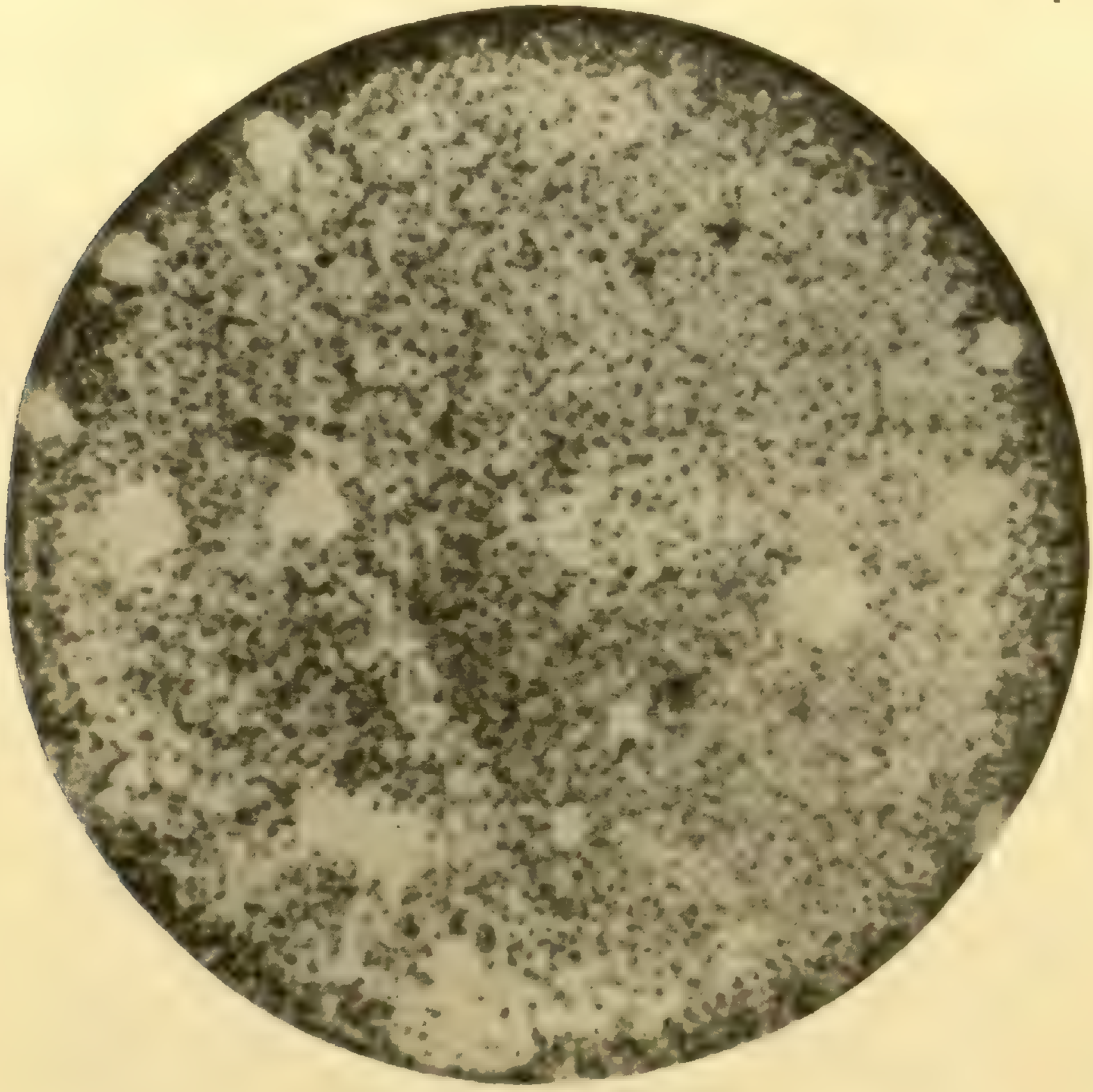

FIG. 5.—*Volutella fructi* S. & H., showing effect of thick sowing.

contact were sporing. Growth then stopped. In the plates bearing only one spore per square millimeter the colonies continued to enlarge slowly and to produce many spores in the central portion, though remaining white, not attaining the usual yellow color. Deep colonies appeared like the superficial, but bore no spores. On February 7 the colonies on thin plates (1 per square millimeter) had attained a

[2] Later study has shown this to be the imperfect stage of an undescribed species of Claviceps, which we shall describe in a subsequent paper.

diameter of 1.5^{mm}. Some of these colonies transferred to tubes continued to enlarge, became tubercular, and developed a yellow center 3 or 4^{mm} in diameter. The whole colony often reached 1^{cm} in diameter. Sister colonies left in the plate (1 per square millimeter) failed so to develop, and it is evident that at even this density normal development is not attained.

The colony is indeterminate in growth, and in plates its size is limited by the presence of adjacent colonies.

SUMMARY REGARDING THE DENSITY FACTOR

This factor produces different effects with the different species. It may inhibit pycnidial formation, resulting in naked spores; it may cause failure to develop color; it may limit the size of the colony; it may be without effect.

There are many paired species of the imperfect fungi agreeing closely, except in the presence or absence of one character. These pairs often occur upon the same host, e. g., *Septoria Lycopersici* Speg. and a Cylindrosporium on the tomato; *Cylindrosporium Chrysanthemi* E. & D. and *Septoria Chrysanthemi* Cav. on the cultivated chrysanthemum.[3] Many other instances could be cited. The lack of fixity of such a structure as even the pycnidium throws doubt upon the validity of such species as these and indicates the necessity of close comparative study.

II. Density of mycelium: zone formation

The formation of concentric zones is by many fungi one of the most conspicuous characters shown in culture. These zones may be

[3] VOGLINO, P., Diseases of cultivated chrysanthemums. Malpighia **15**:329–341. **1902**. (Exp. Sta. Rec. **14**:777.)

HALSTED, B. D., Chrysanthemum leaf spot. Amer. Florist **10**:263. 1894. (Exp. Sta. Rec. **6**:311.)

BEACH, S. A., Leaf spot of chrysanthemum. Report of Horticulturist, N. Y. Exp. Sta. Rept. **1892**:557–560.

HALSTED, B. D., Report of fungus disease of plants. N. J. Exp. Sta. Report **1891**:233–340.

SACCARDO, Syll. Fung. **2**:542, *n.* 3497, 3498, 3757.

TUBEUF & SMITH, Diseases of plants 478.

Year Book U. S. Dept. Agr. **1906**:507.

New York Agr. Expt. Sta. Rept. **14**:529.

New Jersey Agr. Expt. Sta. **1894**:361.

due to any one of many structural characters of the colony; to varying density of spore massing, grouping of pycnidia, mycelial branching, color, etc. It is a frequent phenomenon in nature in the fairy rings of the toadstools, the concentric markings of many leaf spots, fruit rots, etc. These effects have been attributed to various causal agencies, to light relation,[4] to nutrients,[5] to agencies other than light, probably food, to resting periods (HEDGECOCK, *l. c.*), and to mycelial crowding.[6]

ASCOCHYTA CHRYSANTHEMI STEVENS

With the fungus in question the fact that the zones are not due to light or temperature relations is apparent from the fact that they do not coincide with the fluctuations of these two factors (*fig. 6*). In the colony shown, which is that of a plate culture kept at room temperature, there was daily change from warm to cool, light to dark; yet the number of rings does not coincide with the number of these changes; moreover, zones were produced in precisely the same way on plates kept constantly in the dark as on plates kept all of the time in the light, and still the same on plates kept three days in the dark and then three days in the light.

Microscopic examination shows that with this fungus the dark zone is due to a larger number of mycelial filaments, the light zone to a smaller number of threads, as is shown diagrammatically in *fig. 7*. It seems that with this fungus the dense crowding of the filaments, resulting from their repeated branching, inhibits growth either by the products of metabolism or exhaustion of nutriment. There is then a period of quiescence, followed by onward growth of a few scattered hyphae. As these outgrowing hyphae reach beyond the inhibiting influence, they branch repeatedly, until a new dense zone

[4] MOLZ, EMIL, Ueber die Bedingungen der Entstehung der durch *Sclerotinia fructigena* erzeugten Schwarzfaule der Aepfel. Cent. Bakt. **17²**:175.

HUTCHINSON, H. B., Ueber Form und Bau der Kolonieen niederer Pilze. Cent. Bakt. **17²**:602.

HEDGECOCK, G. G., Zonation in artificial culture of Cephalothecium and other fungi. Rept. Mo. Bot. Garden **17**:115–117. *pls. 13–16.* 1906.

[5] MILBURN, THOS., Ueber Aenderungen der Farben bei Pilzen und Bakterien. Cent. Bakt. **13²**:257.

[6] ISTVÁNFFI, GY. DE, Études microbiologiques et mycologiques sur le rot gris de la vigne. Ann. Institut Cent. Ampél. Roy. Hongrois **1905**:183.

is formed. This process is repeated indefinitely. The rapidity of succession of zones is dependent solely upon the relation which rapidity of branching bears to rapidity of increase in length. Slow lineal

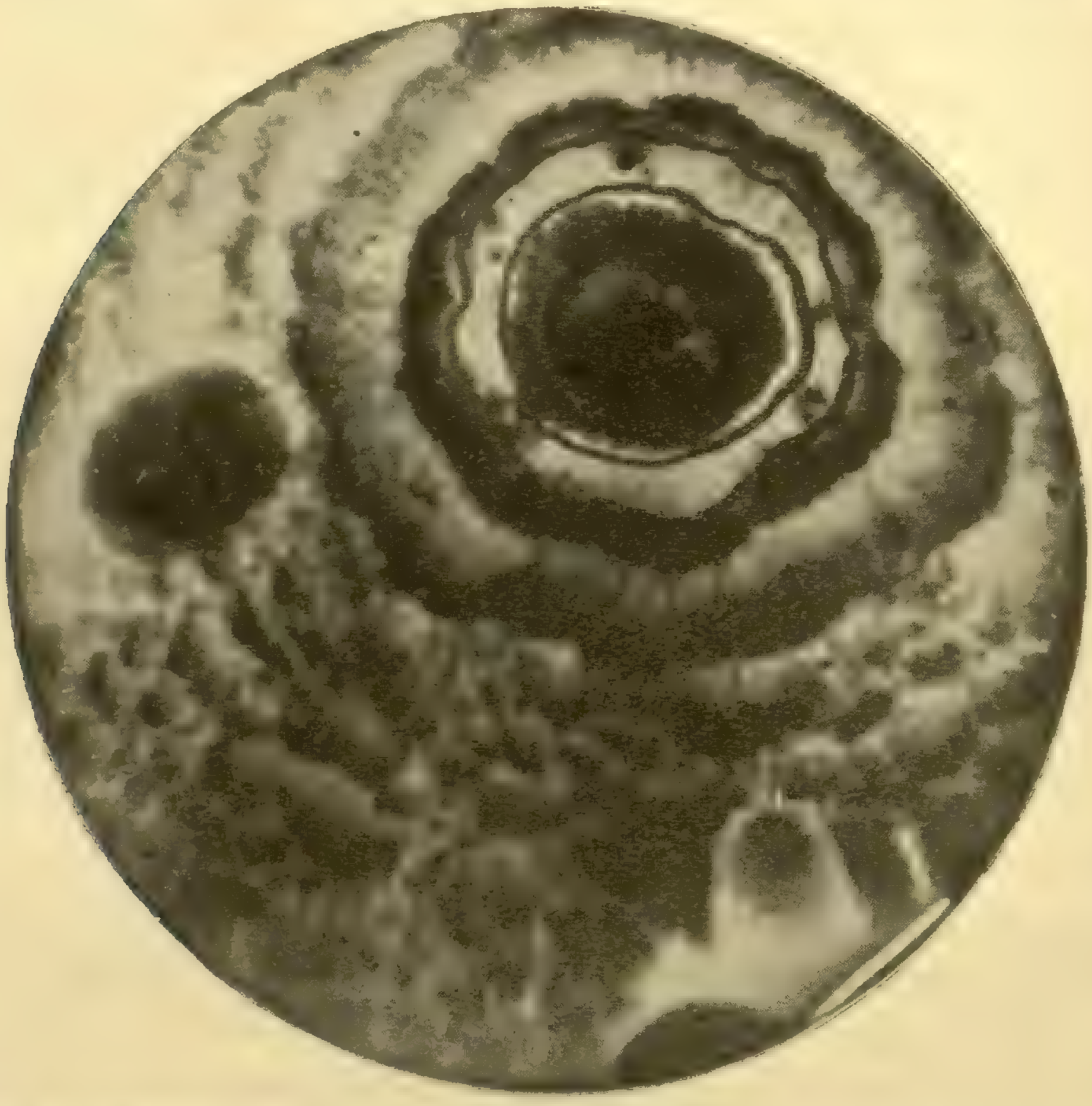

FIG. 6.—*Ascochyta Chrysanthemi* Stevens; plate culture showing that the formation of zones is not coincident with diurnal changes; ink marks show growth for three consecutive days.

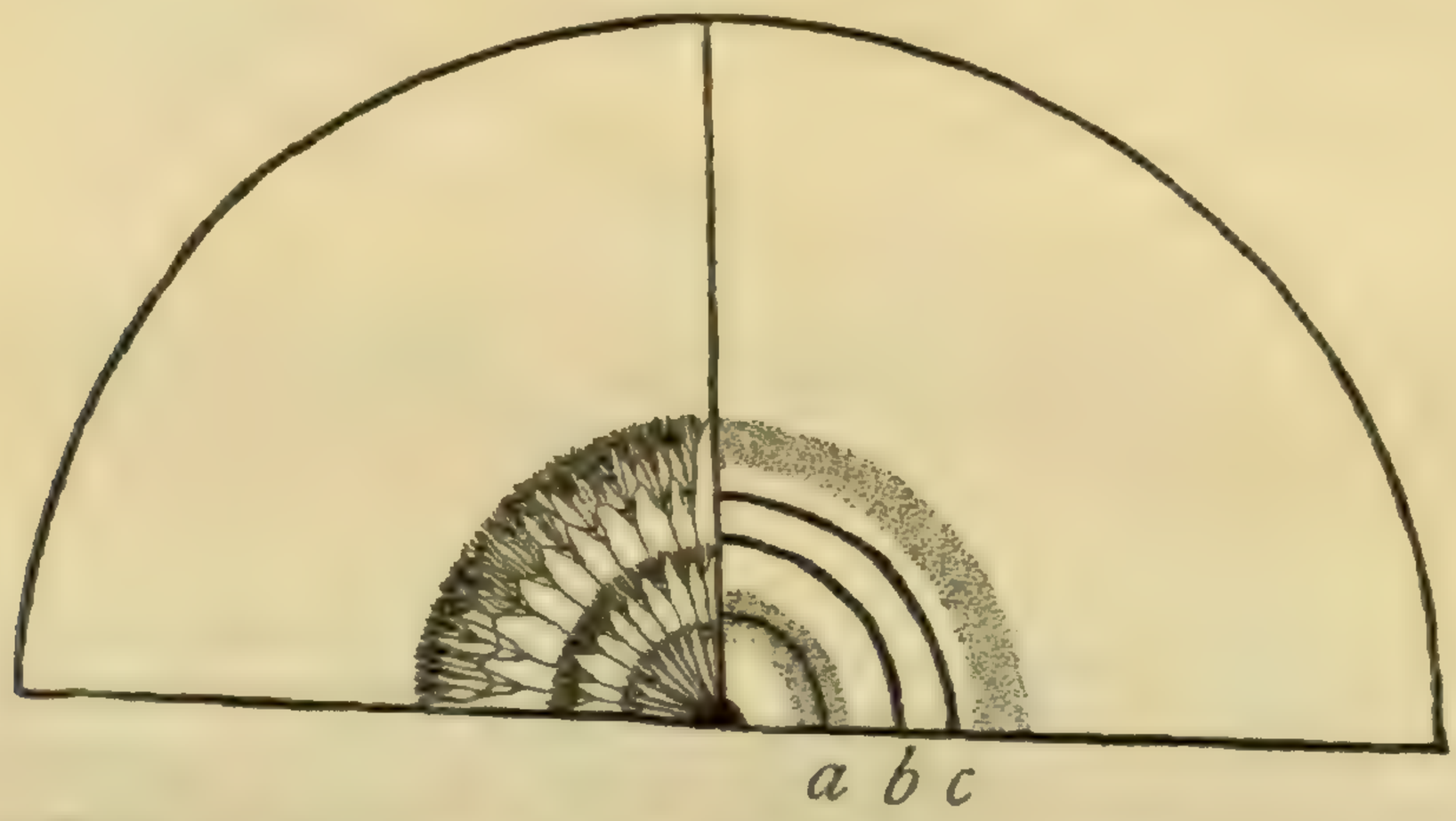

FIG. 7.—Diagram showing, at right, the zones (stippled) and diurnal marks; at left, theoretical expression of cause of zonation.

growth and much branching gives many narrow zones, rapid lineal growth with infrequent branching causes few broad zones.

Sclerotinia Libertiana Fuckel, from lettuce

Zonal sclerotial formation is exhibited by this fungus (*fig. 8*). That this phenomenon may be attributed to crowding of the mycelium is indicated by the fact that adjacent colonies form more sclerotia at their points of contact (*fig. 9*).

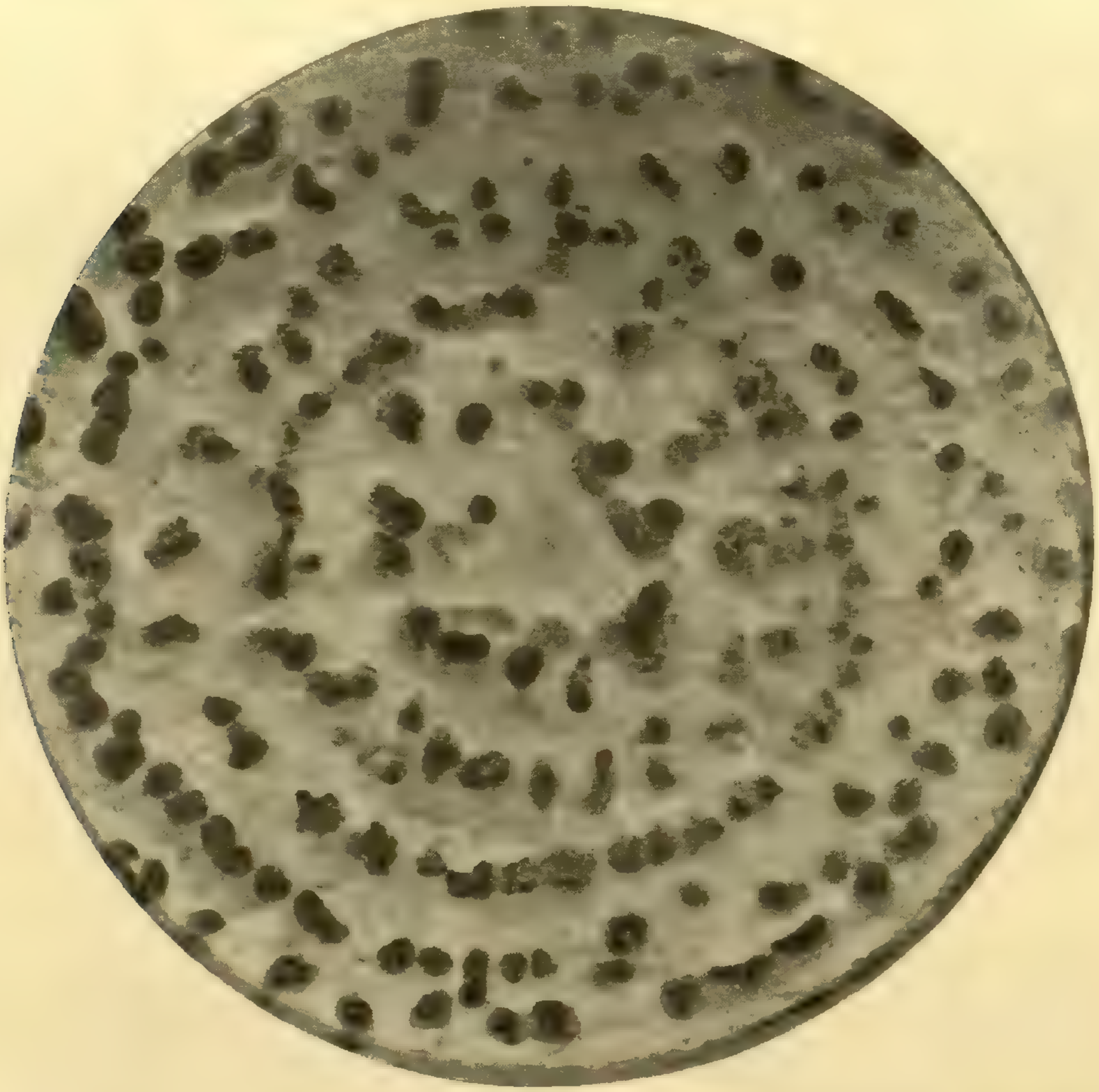

Fig. 8.—*Sclerotinia Libertiana* Fuck., showing zonal formation of sclerotia on corn-meal culture.

SUMMARY REGARDING DENSITY OF MYCELIUM

Zone formation in *Ascochyta Chrysanthemi* is due to crowding of mycelium, not to light or heat relation. A similar conclusion was reached by Istvánffi[6] regarding the very striking zones shown by Sclerotinia. The same cause may apply also with *Daldinia concentrica* and many other fungi of similar structure.

III. Chemical relations

Chemical relations have been studied with eleven fungi, the fungus being usually grown in agar with varying nutrients added. Occasionally other media were used. A chemical base agar (CBA) was

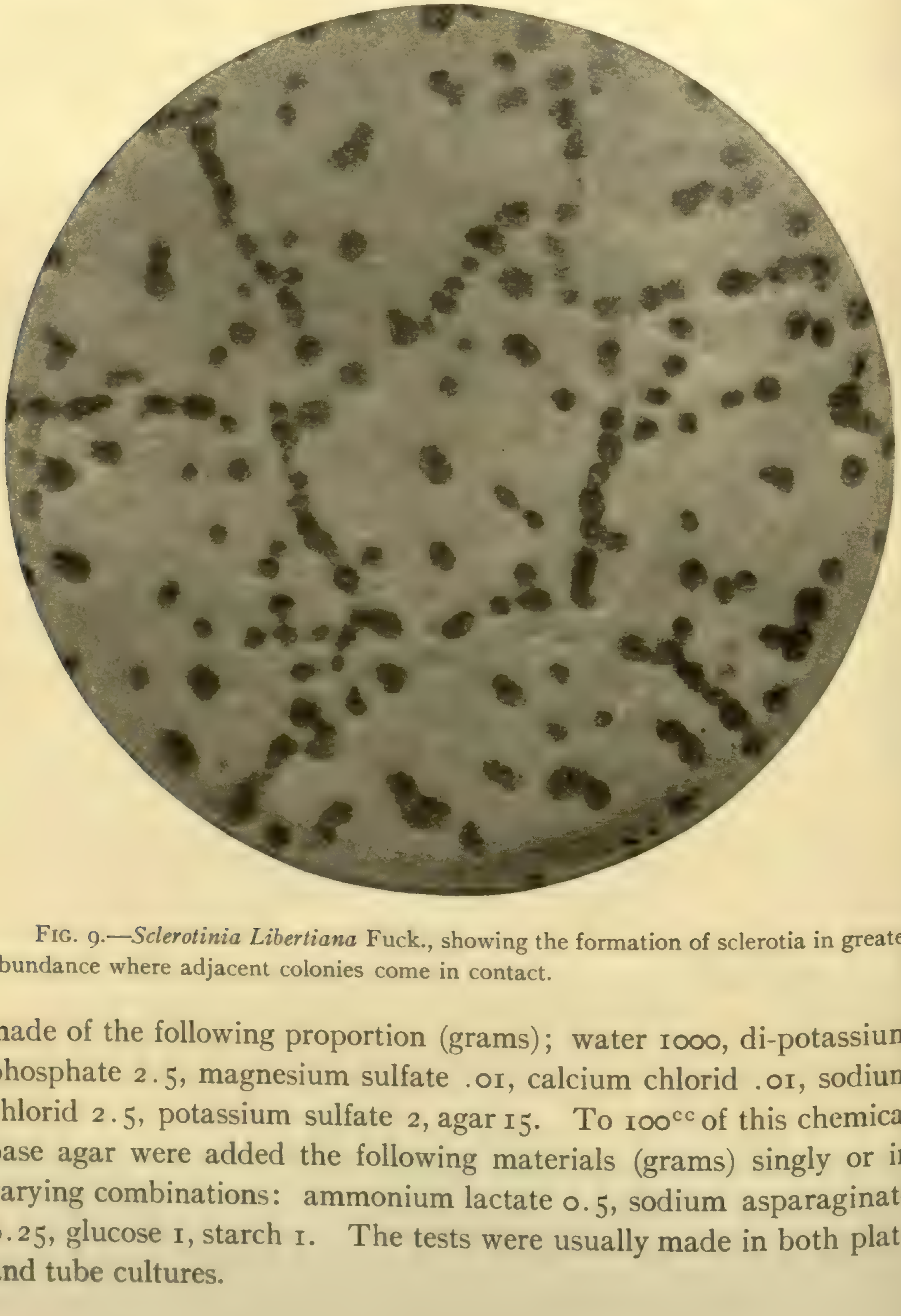

FIG. 9.—*Sclerotinia Libertiana* Fuck., showing the formation of sclerotia in greater abundance where adjacent colonies come in contact.

made of the following proportion (grams); water 1000, di-potassium phosphate 2.5, magnesium sulfate .01, calcium chlorid .01, sodium chlorid 2.5, potassium sulfate 2, agar 15. To 100^{cc} of this chemical base agar were added the following materials (grams) singly or in varying combinations: ammonium lactate 0.5, sodium asparaginate 0.25, glucose 1, starch 1. The tests were usually made in both plate and tube cultures.

VOLUTELLA FRUCTI S. & H., FROM APPLE

This fungus, when sown thin, forms large indeterminate colonies, often with numerous scattered tubercular blotches (*fig. 10*).

On pure agar and CBA the colonies were pale, mycelium hyaline, black tubercles very sparse.

On pea agar, black tubercles were much more abundant, otherwise as on pure agar.

On CBA+sodium asparaginate, black tubercles were still more numerous.

On CBA+sodium asparaginate + starch, black tubercles were more numerous than in any of the above, and the colony was black (*fig. 11*).

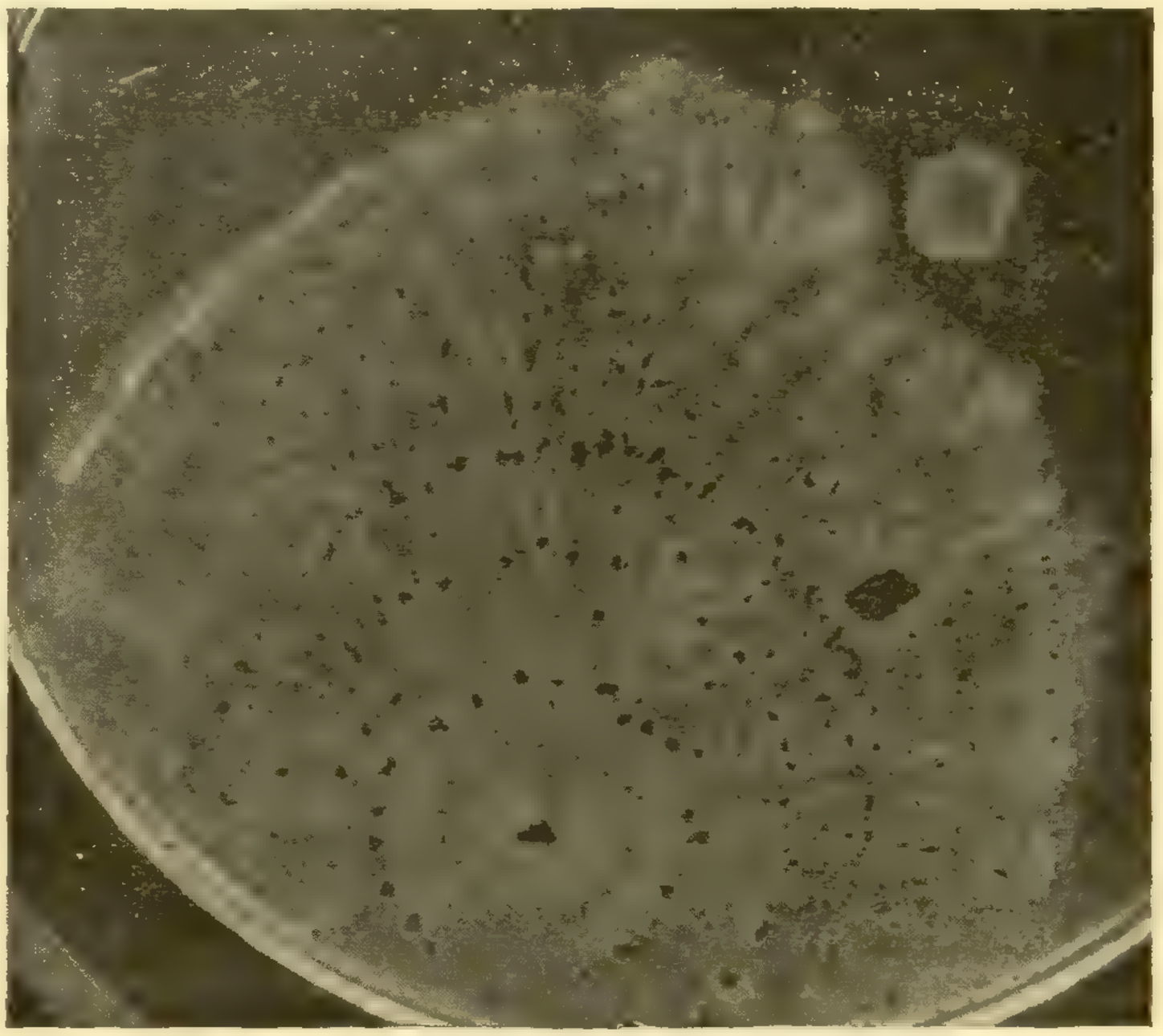

FIG. 10.—*Volutella fructi* S. & H.; colony on pea agar showing tubercular blotches, some of them in concentric rings; mycelium nearly hyaline, due to lack of carbohydrates.

On CBA+sodium asparaginate+glucose, black tubercles were still more numerous, so many as to be contiguous, and the whole colony was densely black.

On gelatinized starch, and starch+Uschinsky's solution, the mycelium was black and some digestion of the starch was observed.

On none of the above media were spores formed, but on sterilized apple twigs spores were produced in abundance.[7]

The differences here noted upon these different media are sufficient to alter entirely the general appearance and to shift the fungus from the Tuberculariaceae-Dematiae to the Tuberculariaceae-Mucedinae.

CONIOTHYRIUM FUCKELII SACC., FROM APPLE

This fungus when growing upon a medium rich in starch becomes black in its peripheral layer. Glucose fails to produce the same

[7] N. C. Agric. Exp. Sta. Bull. 196. June, 1907.

result. The mycelium is hyaline when on pea agar, but tawny on apple agar.[8]

SEPTORIA PETROSELINI VAR. APII, FROM CELERY

This fungus fails to produce naked spores when sown thickly on celery agar though it does so under similar conditions when upon lettuce agar.

COLLETOTRICHUM CARICA S. & H., FROM FIG

This fungus upon the different media used showed striking differences in: number of setae, varying from none to abundant; number of spores, varying from few to many; color, varying from pale to almost black.

FIG. 11.—*Volutella fructi* S. & H.; two black colonies upon CBA + sodium asparaginate + starch.

On CBA growth was scant; acervuli small, setae absent.

On CBA+ammonium lactate and CBA+sodium asparaginate, growth was about as in CBA, except that numerous black setae were present.

On CBA+ammonium lactate+ starch, the acervuli were larger, more numerous, with numerous large black setae.

On CBA+sodium asparaginate+ ammonium lactate, there were a few setae.

On CBA + sodium asparaginate + glucose, black setae were numerous.

EPICOCCUS SP., FROM APPLE AGAR IN PETRI DISHES

This fungus on pure agar and CBA was colorless. On CBA + starch or CBA+glucose, there was much richer mycelial development, which moreover took on a rich yellow color that in spots turned to

[8] N. C. Agric. Exp. Sta. Bull. 196:51.

pink. Sometimes black spots developed on the first of these media but not upon the second. This fungus shows strikingly the differentiating value of starch and glucose for fungus culture.

Upon apple agar still another character developed, a rich golden color of the abundant, floccose, matted, aerial hyphae. This reaction is fully as striking as the familiar rose color produced by certain species of Fusarium.[9]

With this fungus we have absence of color in agar and CBA, but rich coloring, of varying hues, in the presence of carbohydrates and upon apple agar.

Phyllosticta sp., from apple agar in Petri dishes

This fungus grew faster on agar than on CBA, formed pycnidia sparsely on agar and not at all on CBA.

With sodium asparaginate added the mycelium became very dense, with considerable aerial development, remained colorless, and produced few pycnidia, and these visible only with the two-thirds objective. The presence of glucose led to exceedingly profuse pycnidial development, while on starch the growth was as with CBA+sodium asparaginate, showing again the ability to utilize glucose but not starch.

Alternaria sp., from Lawson carnation

This fungus, the cause of an apparently undescribed carnation disease which will be the subject of a subsequent paper, was isolated during October, 1908. There were striking differences in the color of the colony upon different media, varying from merely hyaline to dense black. The size and color of the spores were also so modified as to give much more than what is usually regarded as a specific difference.

On pure agar, CBA, CBA+ammonium lactate, CBA+sodium asparaginate, and upon CBA+ammonium lactate+sodium asparaginate, the mycelium was colorless and the colony correspondingly colorless; while upon CBA+sodium asparaginate+starch and CBA +sodium asparaginate+glucose, the mycelium was very dark, more profuse, more freely branched, and the colony therefore of an entirely different aspect.

9 Bessey, Ernst, Ueber die Bedingungen der Farbbildung bei Fusarium. Inaug. Diss. Halle. 1904.

Spore formation proceeded sparingly, though evenly and regularly, upon pure agar, CBA, CBA+ammonium lactate, CBA+sodium asparaginate, CBA+sodium asparaginate+ammonium lactate; but very abundantly upon CBA+sodium asparaginate+starch and upon CBA+sodium asparaginate+glucose. Here the sodium asparaginate seems not to furnish the carbon in sufficiently available form, though starch or glucose do so to nearly equal extent.

The size, color, and septation of the spores were also greatly influenced by the medium. From carnation-agar plates the spores measured 16 to 52 μ long by 6 to 13 μ thick, bearing 0 to 3 longitudinal septa and three to seven transverse septa; while from the live carnation leaf the spores were 26 to 123 μ long by 10 to 20 μ thick, bearing 1 to 9 or often numerous longitudinal septa and 3 to 15 transverse septa. It is seen that the spores are approximately twice as long, twice as thick, of darker color, and with many more septa in each direction upon the natural medium than upon the carnation agar, differences which would ordinarily be regarded as clearly of specific rank.

Alternaria Brassicae (Berk.) Sacc., from collard

This fungus made hyaline mycelium in CBA and CBA+sodium asparaginate; black mycelium in CBA+sodium asparaginate+glucose and in CBA+sodium asparaginate+starch, starch producing by far the most pronounced effect.

Digestion of the starch grains, somewhat in advance of the tips of the oncoming fungous threads, produced a clear zone surrounding each colony in the starch-bearing plates.

Ascochyta Chrysanthemi Stevens

This fungus was grown in the usual media with no significant effects, except that the fungus did not digest the starch grains afforded in the medium.

A deposit of great thickness around mycelial threads was made in the case of certain media and not in others, as has already been noted.[10]

In some instances culture at a high temperature occasioned this same reponse.

[10] Bot. Gazette 44:241. 1907.

SUMMARY OF CHEMICAL RELATIONS NOTED

The most striking response to chemicals is in color, which so far as observed was invariably heightened by the presence of chemicals bearing carbon in available form, the form of available carbon varying for different fungi. Some fungi, possessing ability to digest starch, can utilize this as a source, while to others the carbon of starch is inaccessible. Special unknown chemicals in apple add vivid colors to fungi otherwise hyaline. Some chemicals also promote or inhibit spore formation. Some inhibit or promote growth of setae and some even alter the size, color, and septation of spores. MILBURN,[5] working under KLEBS, has also noted pronounced effects of chemicals upon the color of fungi. The difference in color effects produced by different fungi under the same conditions, and with the same fungus under different conditions, is also noted by BESSEY.[9] No correlation is noted between rapidity of lineal growth and nutritive value of the medium. In many instances most rapid lineal growth occurred in what was surely the poorest medium. Very poor media suffice in many cases also for spore formation, while rich media often result in cessation of spore formation.

Colletotrichum Lindemuthianum, sometimes with setae, often without, has long been of questionable generic position. The same is true of several other species of this genus. *Alternaria Brassicae* and *Macrosporium Brassicae* agree closely except as to presence or absence of catenulate spores.[11] Variation of this kind is probably due to variation in chemical composition of the supporting medium, e. g., change in sugar content as ripening proceeds, acting in such way as to give the fungus the appearance of belonging to one genus when upon the green sugar-tree fruit, to another genus as the starch gives place to sugar as the fruit ripens.

IV. Light relation

The absence of material effect of light upon lineal growth with these species of fungi is shown in Table I.

Ascochyta Chrysanthemi Stevens.—The growth is more floccose in darkness.

Phyllosticta sp.—This fungus forms its pycnidia in beautiful con-

[11] *A. Brassicae* "hyphis brevibus conidiis 60–80 × 14–18 μ, 6–8 septato-muralibus." *M. Brassicae* "hyphis obsoletis conidiis 50–60 × 12–14 μ, 5–11 septatis."

centric rings when in open room, i.e., alternate light and darkness, but in continuous darkness they were irregularly scattered. Culture no. 35 made concentric rings when in the light, and failed to do so

TABLE I

Relation of light to growth

Figures express growth in millimeters. The cultures marked "alternate" were kept several days in light and several days in dark; *L* light; *D* dark. Inoculated Dec. 8, 1908.

	Light condition	December										
		9	10	11	12	13	14	15	16	17	18	19
Macrosporium Brassicae	In light	germ	1		6	9	13	16	17	23	26	28
	Alternate	*L*	*L*	*L*		*L*	*D*	*D*	*D*	*L*	*L*	*L*
	light & dark	germ	1		6	10	12	15	17	23	26	28
	In dark	germ	1		6	9	11	15	17	21	29	29
Phyllosticta sp.	In light	o	gr.		4	7	10	13	14	16	20	23
	Alternate	*L*	*L*	*L*	*L*	*L*	*D*	*D*	*D*	*L*	*L*	
	light & dark	o	gr.		4	7	10	13	13	16	18	20
	In dark	o	gr.		4	6	7	10	13	16	19	20
Ascochyta Chrysanthemi	In light		4		12	15	17	25	26	33	39	41
	Alternate	*L*	*L*	*L*	*D*	*D*	*D*	*D*	*D*	*L*	*L*	*L*
	light & dark	2	3		12	16	20	25	30	37	39	45
	In dark				12	14	18	22	25	31	37	37

when moved to darkness. Cultures kept in the open room lay down rudiments of pycnidia mainly during the night, and it is probable that light exerts enough inhibiting influence on pycnidial development to give a growth predominance during the day and a fructifying predominance during the night (HEDGECOCK, *l. c.*[4]).

Alternaria Brassicae (Berk.) Sacc.—With this fungus the end of each day's growth, evening, marks the edge of a zone. The zone thus marked is intensified during the succeeding twenty-four hours by color changes. While zones are formed to some extent in continued darkness, they are more pronounced in the room condition.

SUMMARY OF LIGHT RELATION

Light exerts little or no effect upon lineal growth with these fungi. It appears to exert an inhibiting influence on pycnidial development and in some instances is the cause of zonation in colonies.

V. Unknown factors

Ascochyta Chrysanthemi Stevens

This fungus frequently exhibited differences in character along different radii of the same colony, the conditions of medium, thickness of sowing, humidity, etc., being apparently identical.

Fig. 12 shows such a colony. Along the radius *aa*, at *b*, the colony bore pycnidia abundantly, and the mycelial progeny of this strain extending to the periphery of the colony was rich in pycnidia, while

Fig. 12.—*Ascochyta Chrysanthemi* Stevens, showing abundant pycnidia on radius *aa*, at the point *b*, and paucity of pycnidia elsewhere.

most other radii of the colony were sterile or nearly so. Transfers were made from the point *c* (sterile) and *d* (pycnidial) to fresh plates. The sterile mycelium produced a colony which was sterile through its early days. As it aged it formed a few large pale pycnidia. The fertile strain produced a fertile colony with very numerous, though

small, pycnidia. Transfers made again from these two strains resulted in a complete reversal of character, the fertile becoming sterile and the sterile becoming fertile. No explanation of this suggests itself.

When this fungus was plated from a suspension of spores, two types of colony developed, corresponding to the two strains mentioned

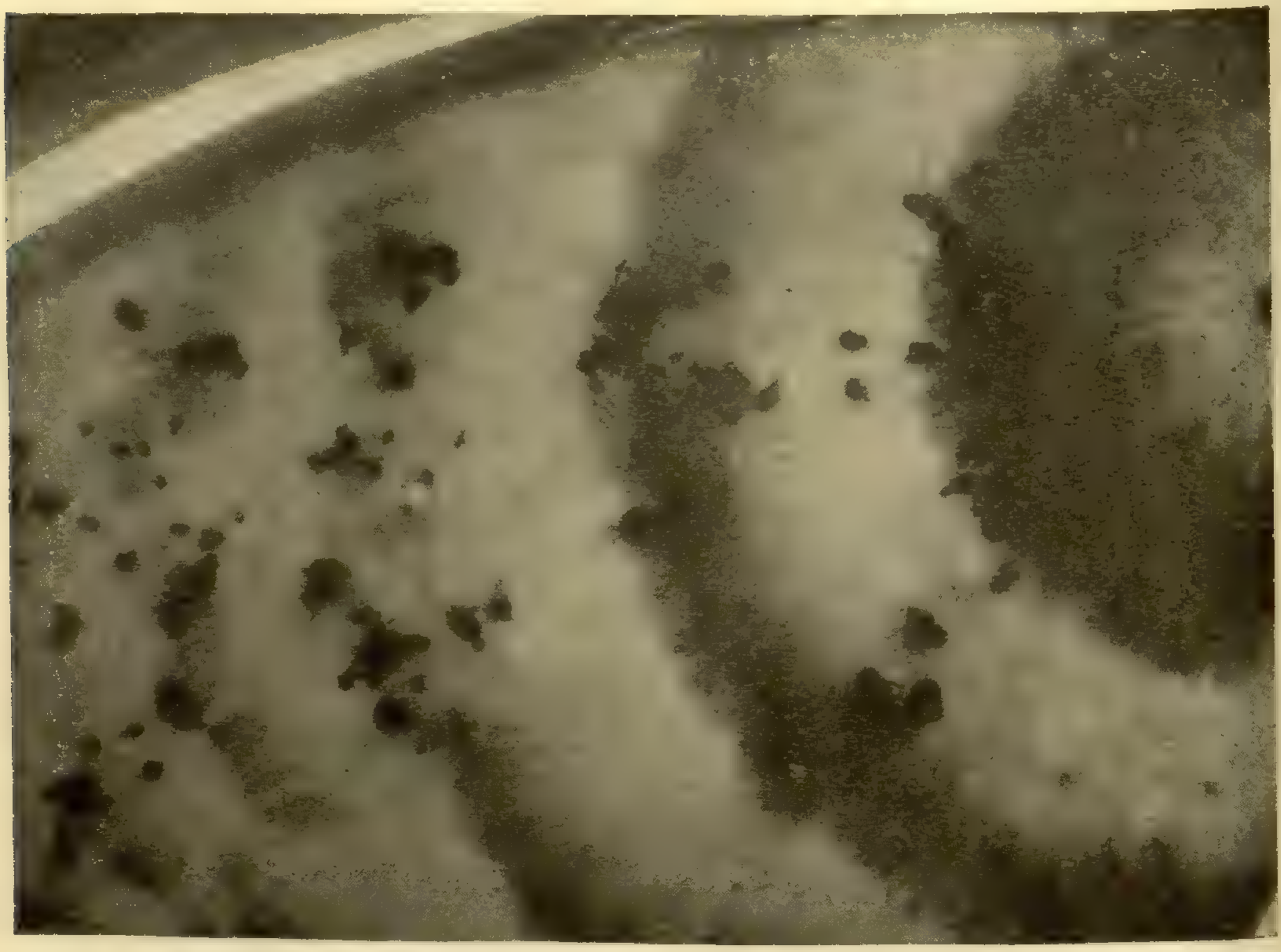

FIG. 13.—*Ascochyta Chrysanthemi* Stevens; portion of colony showing few pycnidia; cf. *fig. 14*.

above. The first "type of few pycnidia" developed a copious aerial mycelium of loose floccose nature, extended regularly in all directions, and was long devoid of pycnidia. When the pycnidia did form, they were few, large, and superficial (*fig. 13*). The second "type of many pycnidia" had little or no aerial mycelium, all the mycelium being either immersed or of strict growth; was roughly circular in colony, not regularly so as in first type; and small, irregular, mostly immersed pycnidia were formed in myriads throughout the colony (*fig. 14*). These two types of colony appeared on the same plates which were inoculated with spores from the same pycnidium. They therefore developed in the same nutrient condition, humidity, temperature, etc. Depth of planting was not the cause of these differences,

since flooding the plate with an extra tube of agar after the agar first plated had set, did not change the proportion of the two types. Nor did sowing in such way that the spores were at the bottom rather than at the top of the agar change results. There was a marked tendency of colonies of both types of the fungus to become more productive of large pycnidia where two different colonies approach each other, sug-

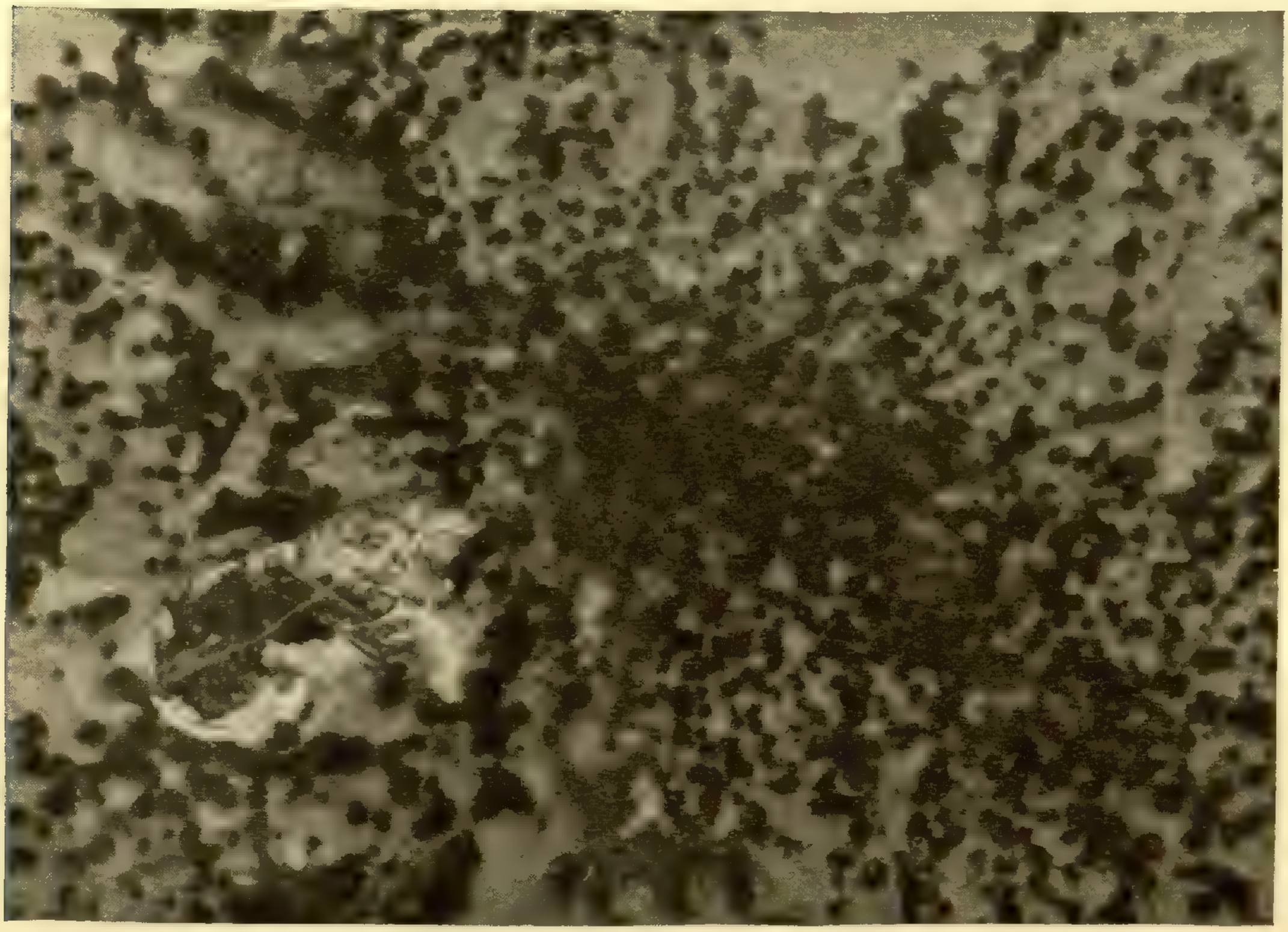

FIG. 14.—*Ascochyta Chrysanthemi* Stevens; portion of colony showing many pycnidia; cf. *fig. 13*.

gesting that there might be needed a cooperation of two diverse strains in order to form a pycnidium; that the strains of few pycnidia lacked the requisite individuals, and that the strains of many pycnidia had more than one individual to the colony. To test this, colonies were traced from the earliest development, resulting in clear evidence that in some instances a colony developed from a single spore was one with few pycnidia; in other instances a single spore produced a colony of many pycnidia.

CONIOTHYRIUM FUCKELII SACC., FROM APPLE

In one instance this fungus, which rarely fruited, made pycnidia in almost perfect circles near the margins of each colony on the plate (*fig. 15*).

These variations are inexplicable and remind one of the mysterious change from the ascigerous to the non-ascigerous condition so frequently met in life-history work with the imperfect fungi.

Variability in spore measurements

Since the beginning of mycology it has been customary to give spore measurements in specific description, probably originally with

FIG. 15.—*Coniothyrium Fuckelii* Sacc.; portions of two colonies showing circles of pycnidia near margins.

the idea of giving some information as to the approximate size of the plant concerned rather than to give exact descriptive limitations. With the advance of time, great importance has come to be attached to spore measurements, greater perhaps than is warranted, and many species are now founded upon divergence in this one character—and often upon slight divergence.

To ascertain the variability in spore measurement under constant conditions and its variability as occasioned by changes in environment, studies with several species of fungi were undertaken.

The measurements were all made in water in which the spores had stood long enough to become fully turgid, taking only such spores as were completely ripe, as was shown by the fact that they were extruded from the pycnidium, ascus, or sporodochium naturally, without assistance. An eyepiece micrometer was used and the units here employed are usually one division of the eyepiece scale (equal to 3.7 μ), which constituted in most cases as small a unit as could be used to advantage. Spore measurements involving half the division were recorded as with the next lower integer unless otherwise designated. To avoid any possibility of unconscious selection, the spore lying closest to contact with the end of the micrometer scale at the completion of a measurement was taken for the next measurement. In the polygons each small square (one 256th of a square inch) represents one spore.

We wish to acknowledge our indebtedness to Dr. G. H. SHULL, who has kindly read this portion of the manuscript, for calculating the constants, and to Mr. B. B. HIGGINS, by whom most of the measurements were made and upon whose very accurate and painstaking work the value of the measurements depends.

ASCOCHYTA CHRYSANTHEMI STEVENS

A. *Spores from the large pycnidium type* (see p. 18)

Pycnidium no. 1. A large pycnidium produced in a colony which had very few pycnidia.

$M = 4.9645 \pm 0.0393$
$\sigma = 0.9787 \pm 0.0278$
C. V. $= 19.714 \pm 0.581$
$n = 284$

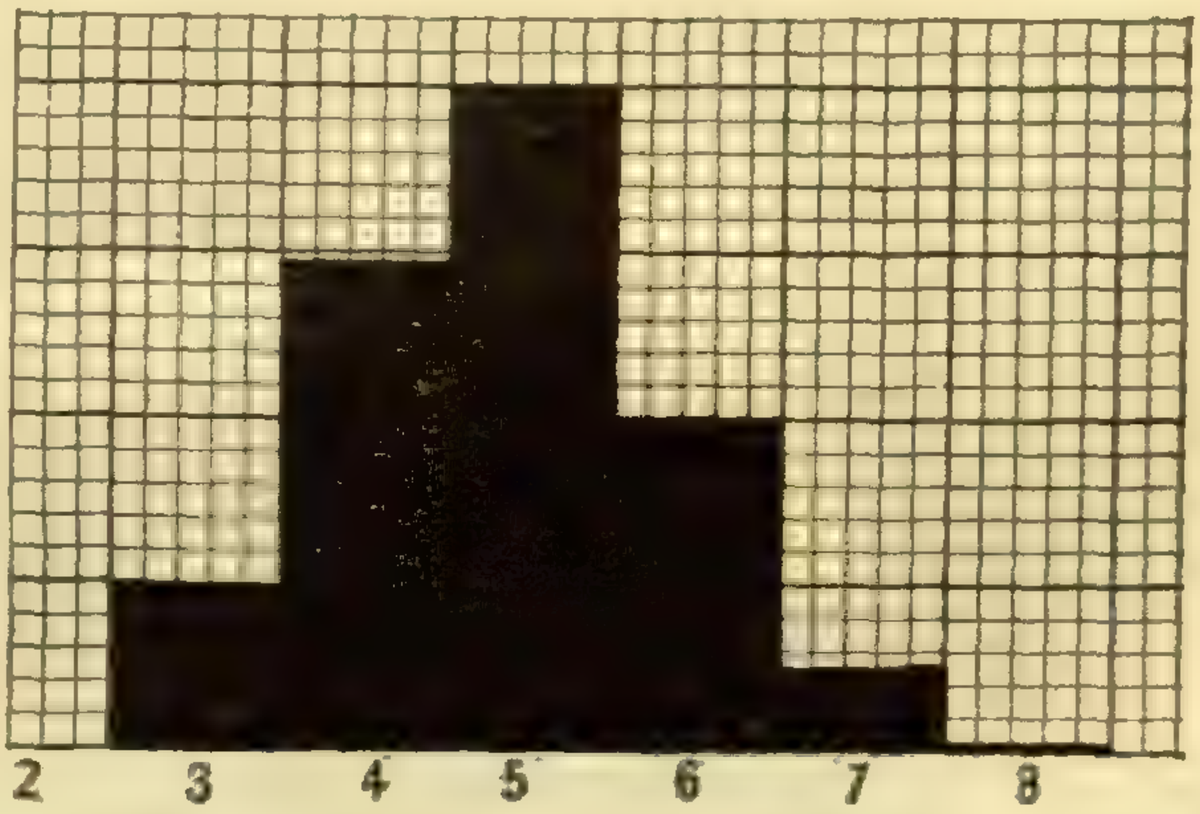

FIG. 16. — *Ascochyta Chrysanthemi* Stevens. Polygon of spores from pycnidium no. 1, large type. 3 should cover 20 squares instead of 25.

Pycnidium no. 2. Large type.

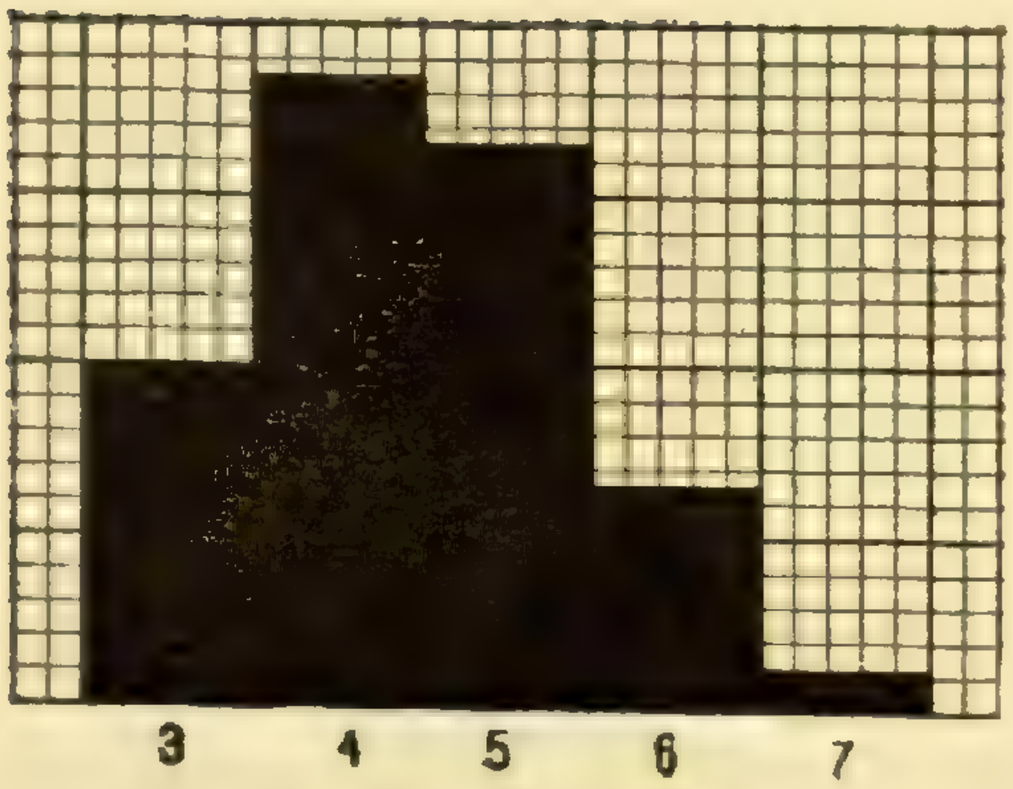

FIG. 17.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores from pycnidium no. 2, large type.

$M = 4.4318 \pm 0.0398$
$\sigma = 0.9589 \pm 0.0281$
C. V. $= 21.638 \pm 0.650$
$n = 254$

Pycnidium no. 3. From a plate bearing one large colony. The whole colony was characteristically one of few pycnidia, which were of large type and light color. The spores were obtained without any possibility of the pycnidium being torn; that is, they were normally ripe spores.

$M = 3.3848 \pm 0.0245$
$\sigma = 0.6714 \pm 0.0173$
C. V. $= 19.836 \pm 0.531$
$n = 343$

It is seen that these three separate pycnidia of the same type gave modes of 4.9645 ± 0.0393, 4.4318 ± 0.0398, and 3.3848 ± 0.0245; or, expressed in terms of the systematist, that in the three pycnidia the spores measured 11.1–29.6 μ, mostly 18.5 μ; 11.1–25.9 μ, mostly 14.8 μ; 7.4–22.2 μ, mostly 11.1 μ; showing that measurements from one pycnidium alone are not sufficient for reliable characterization.

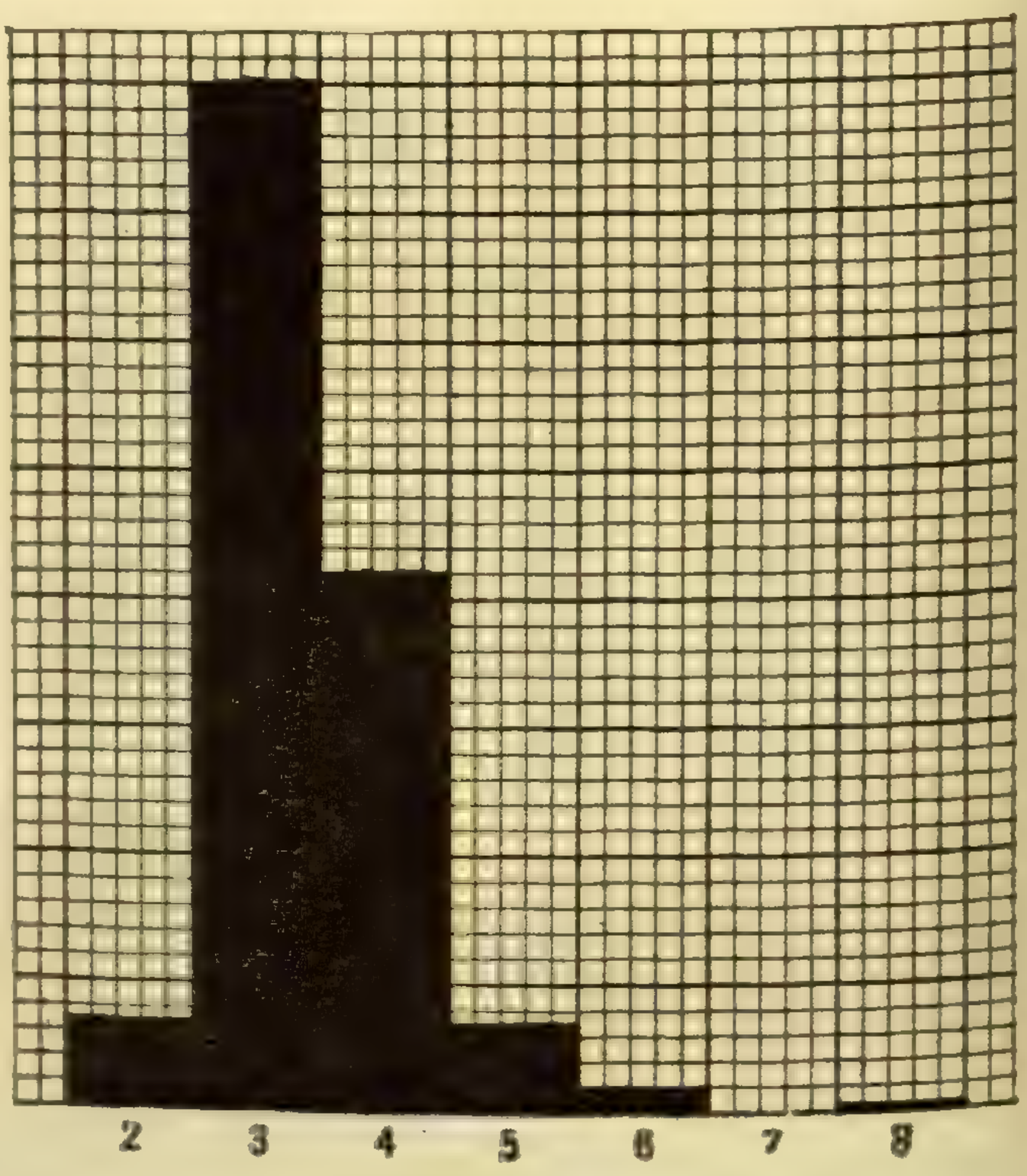

FIG. 18.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores from pycnidium no. 3, large type.

B. *Spores from small pycnidium type* (see *fig. 14*)

Pycnidium no. 4. Small type.

M= 3.6011±0.0363
σ= 0.7183±0.0256
C. V.=19.947 ±0.740
n=178

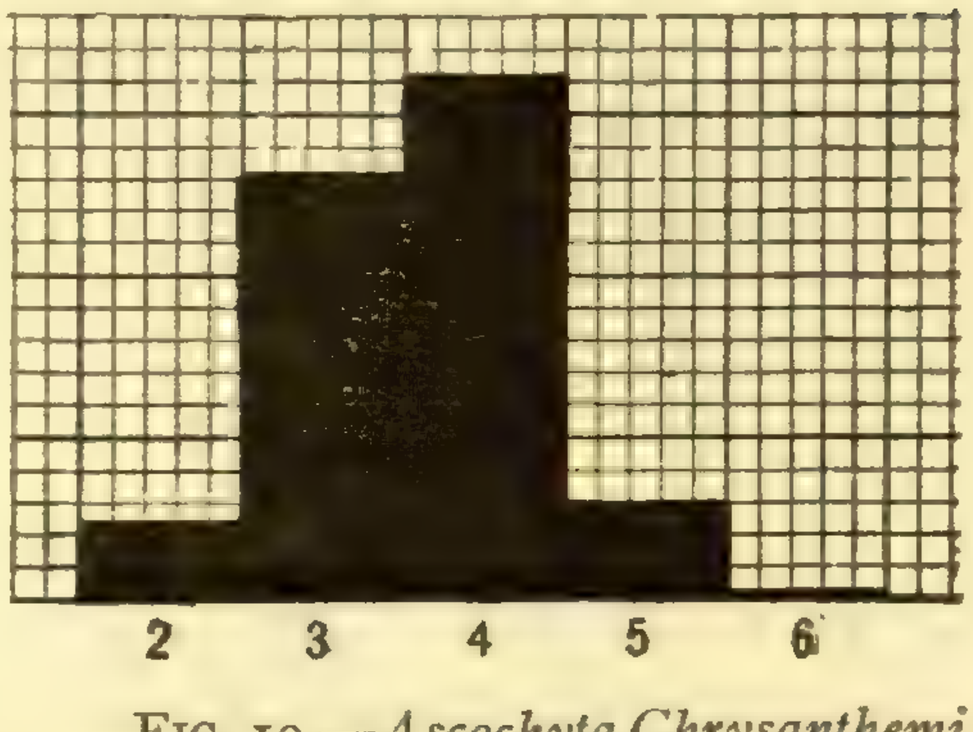

FIG. 19.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores from pycnidium no. 4, small type.

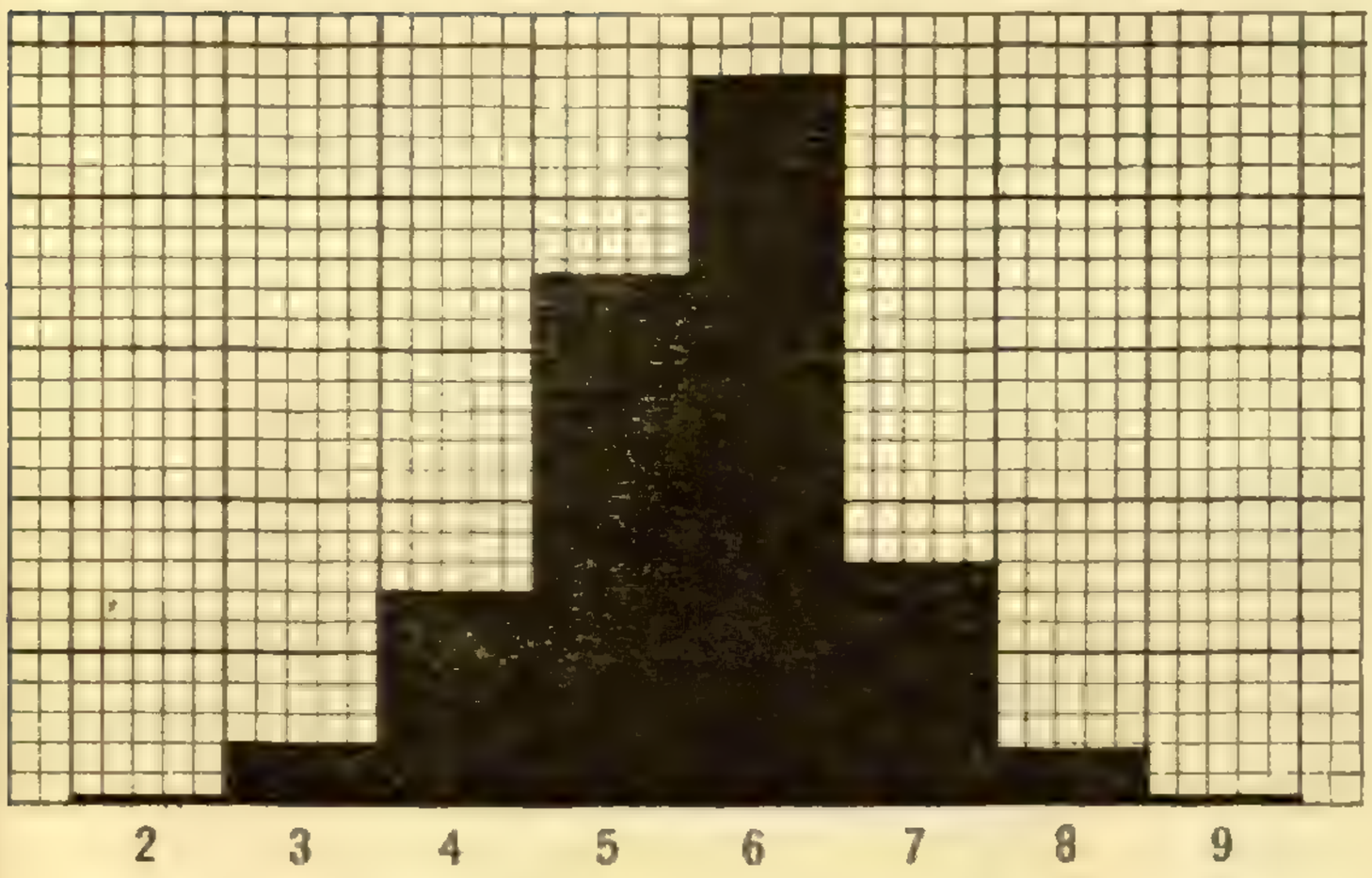

FIG. 20.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores from pycnidium no. 5, small type.

Pycnidium no. 5. Spores taken from small pycnidia from colony shown in *fig. 14*.

M= 5.5850±0.0414
σ= 1.0737±0.0293
C. V.=19.225 ±0.543
n=306

Pycnidium no. 6. A very small pycnidial type.

M= 5.3629±0.0544
σ= 1.2711±0.0385
C. V.=23.702 ±0.756
n=248

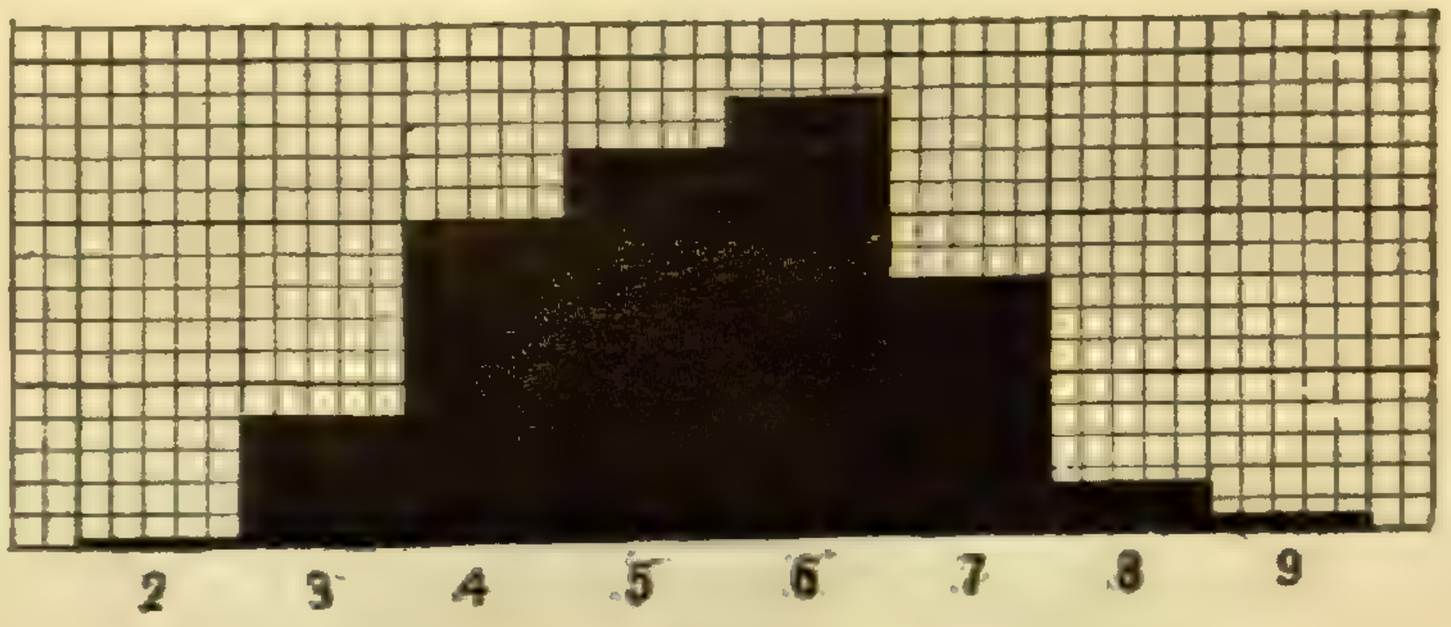

FIG. 21.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores from pycnidium no. 6.

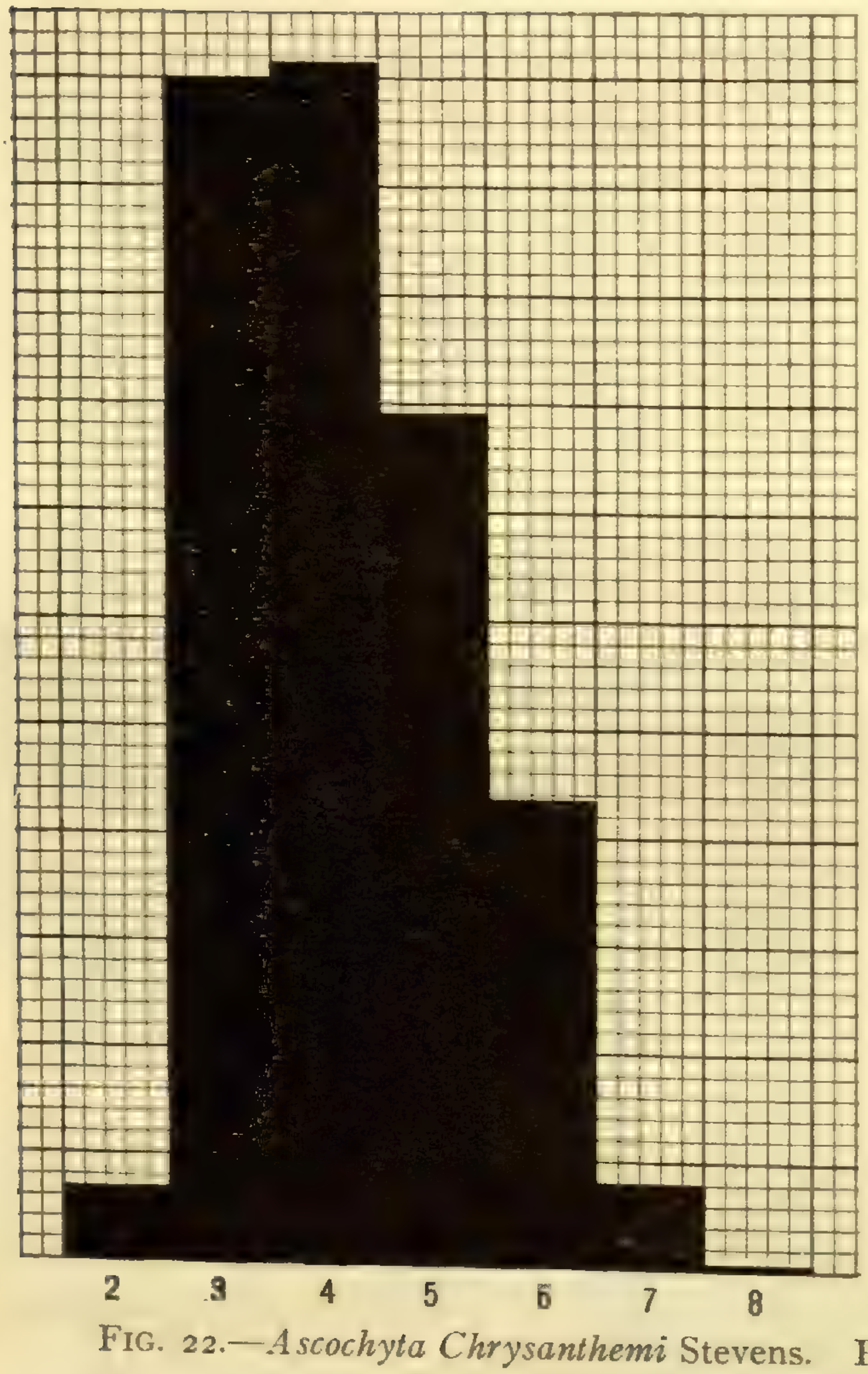

FIG. 22.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores of large pycnidia.

Collecting the data from the large pycnidium type in one polygon, and similarly with the small pycnidium type we have:

$$M = 4.1935 \pm 0.0247$$
$$\sigma = 1.0902 \pm 0.0174$$
$$C.\ V. = 25.998 \pm 0.443$$
$$n = 889$$

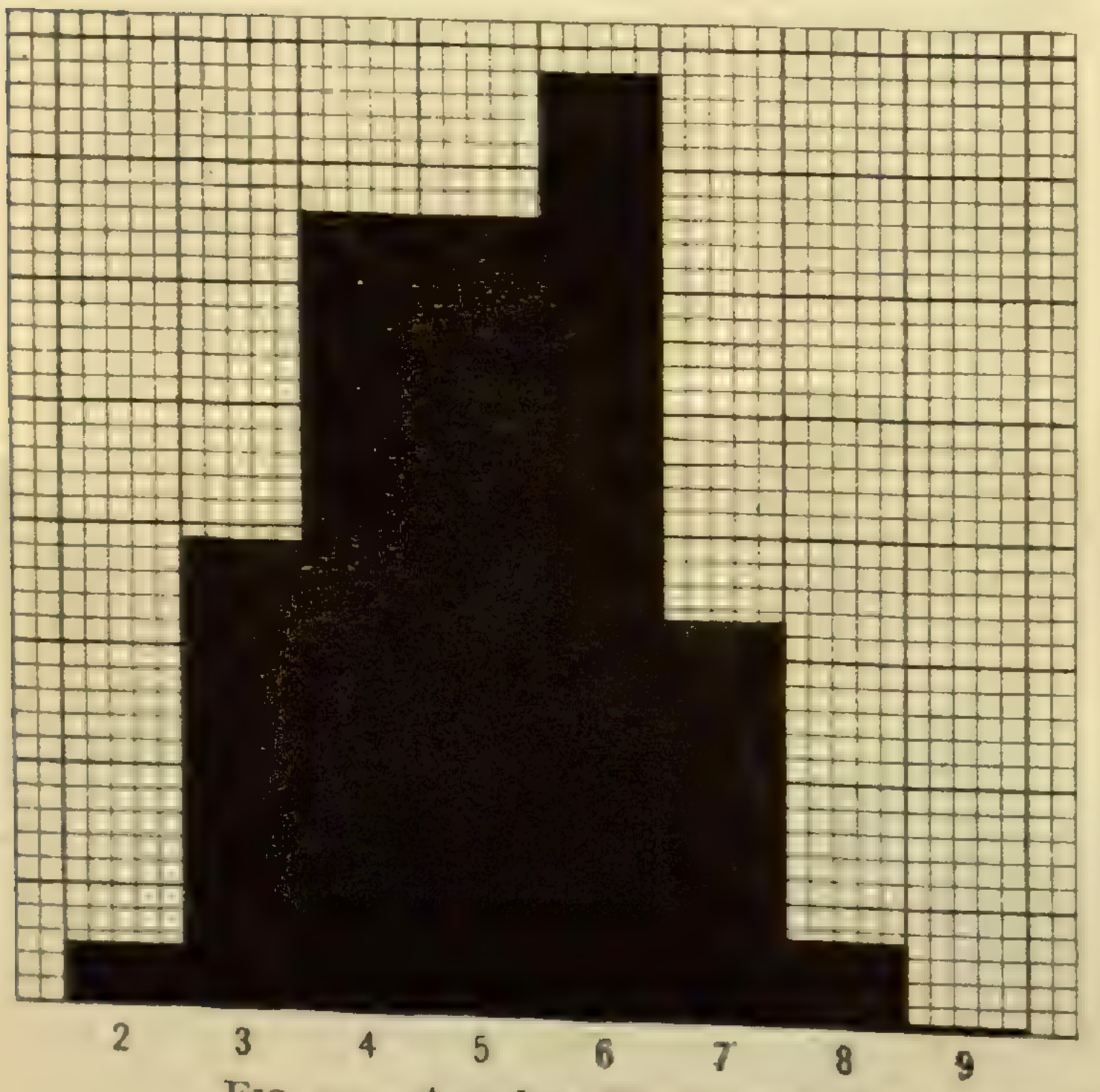

FIG. 23.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores of small pycnidia.

$$M = 5.0379 \pm 0.0335$$
$$\sigma = 1.3492 \pm 0.0237$$
$$C.\ V. = 26.781 \pm 0.503$$
$$n = 738$$

It is seen that there is a tendency throughout for the smaller pycnidia to produce larger spores than are produced by the large pycnidia.

C. *Measurements of spores from different media*

Pure agar. The pycnidia on this plate were very scant, although they were normal in appearance and size.

M= 2.6241±0.0313
σ= 0.5354±0.0221
C. V.=20.402 ±0.878
n=135

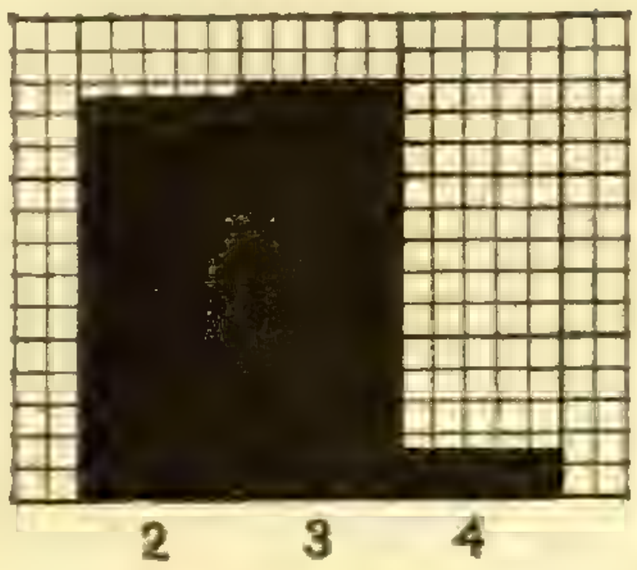

FIG. 24.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores from pure agar.

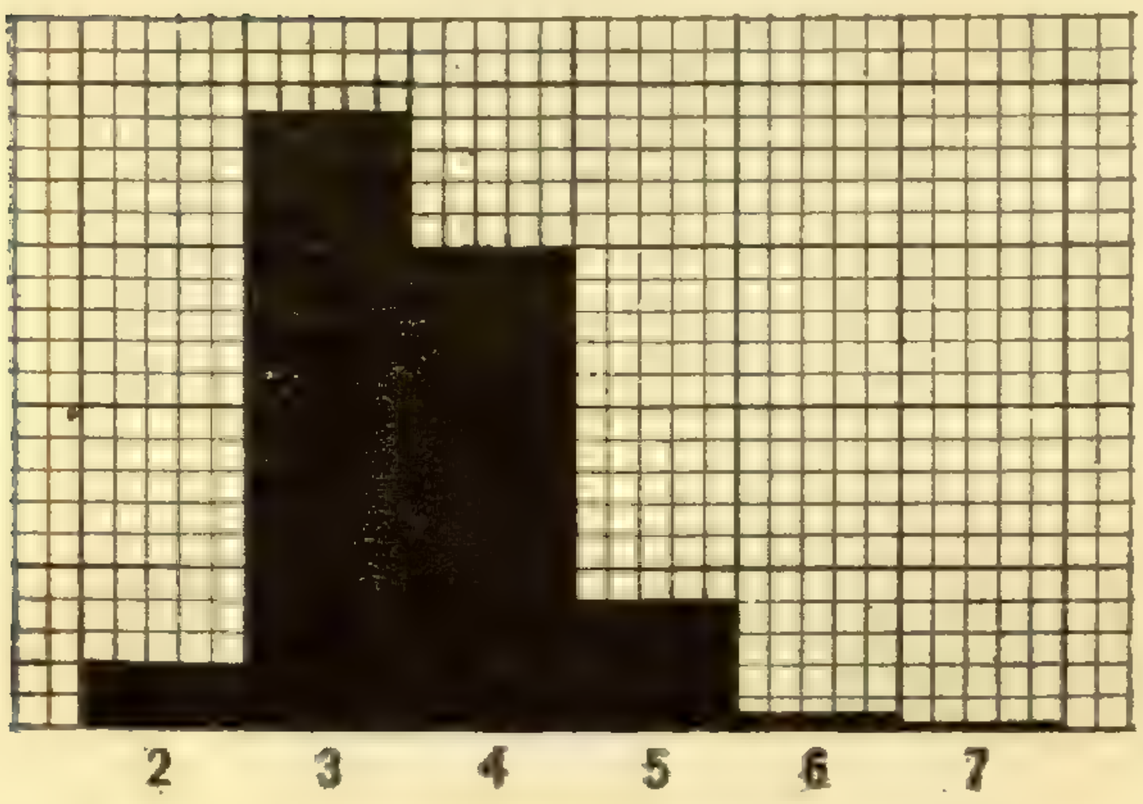

FIG. 25.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores from CBA+0.25 per cent. sodium asparaginate.

CBA+0.25 per cent. sodium asparaginate.

M= 3.5637±0.0358
σ= 0.7579±0.0253
C. V.=21.267 ±0.725
n=204

CBA+sodium asparaginate +starch.

M= 5.4267±0.0355
σ= 0.7896±0.0251
C. V.=14.551 ±0.459
n=225

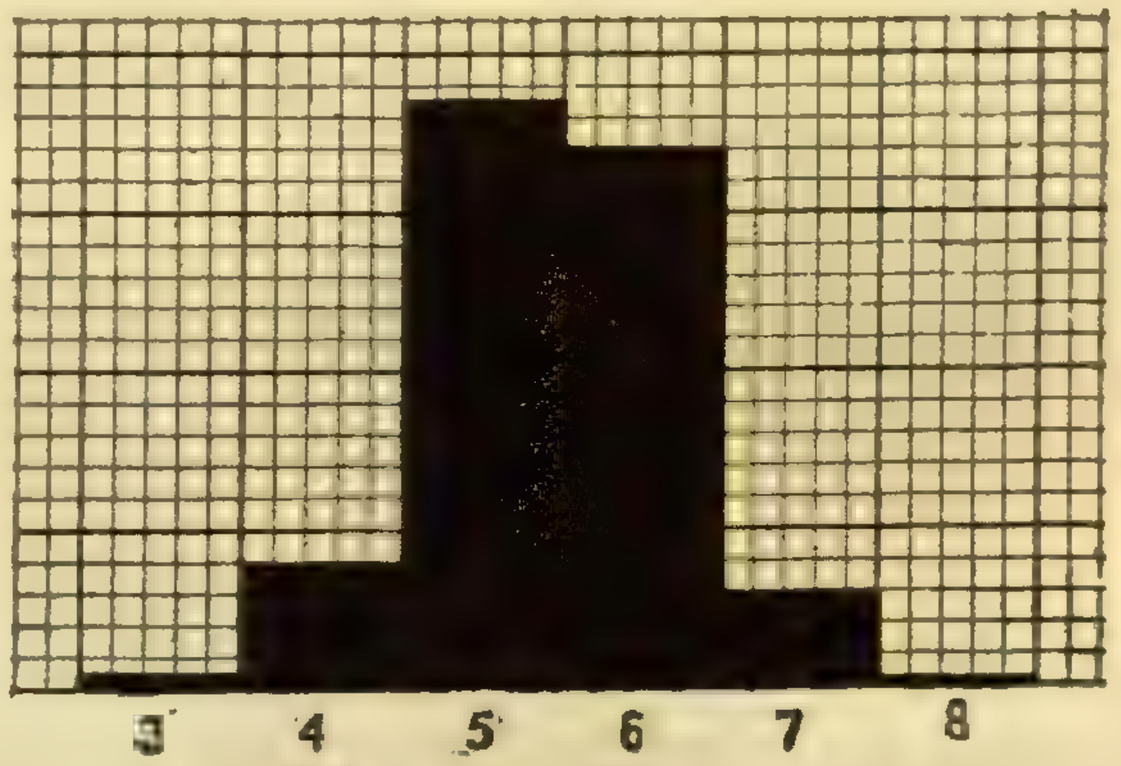

FIG. 26.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores from CBA+sodium asparaginate+starch.

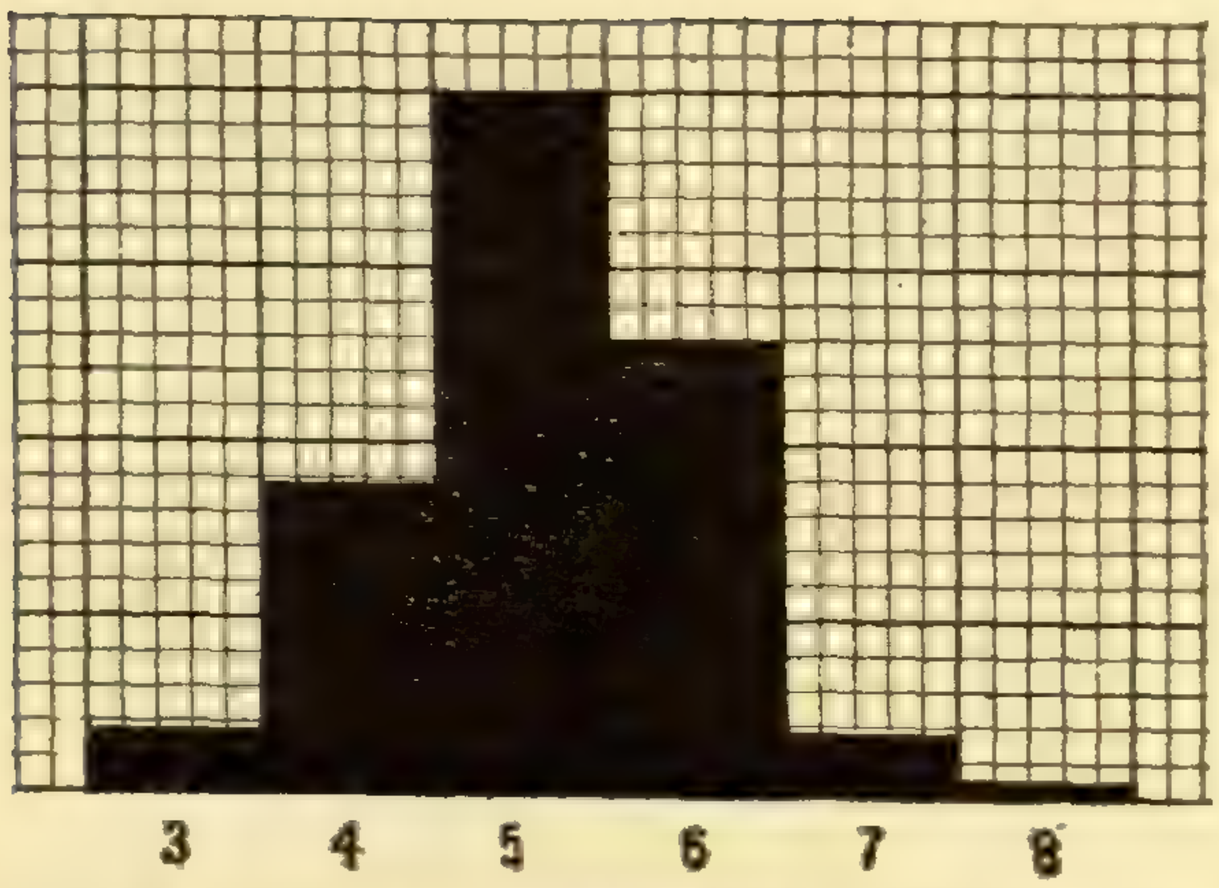

FIG. 27.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores from CBA + sodium asparaginate + glucose.

CBA + sodium asparaginate + 1 per cent. glucose. This was a remarkable colony with spores distinctly smoky or olivaceous.

$$M = 5.1422 \pm 0.0408$$
$$\sigma = 0.9214 \pm 0.0289$$
$$C.V. = 17.919 \pm 0.579$$
$$n = 232$$

Plated thickly in 4 per cent. pea agar.

$$M = 4.3246 \pm 0.0392$$
$$\sigma = 1.0138 \pm 0.0277$$
$$C.V. = 23.442 \pm 0.674$$
$$n = 350$$

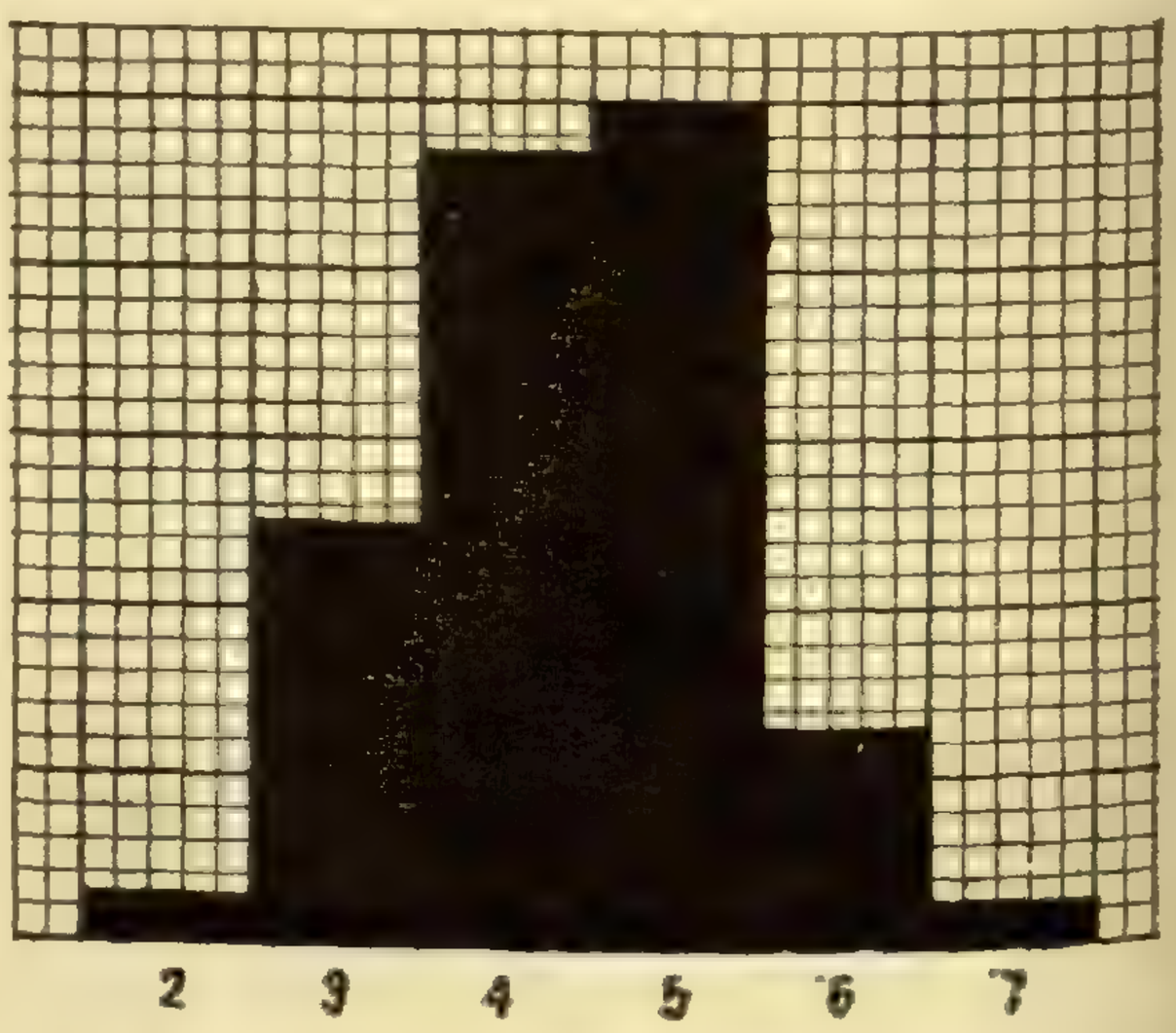

FIG. 28.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores from 4 per cent. pea agar.

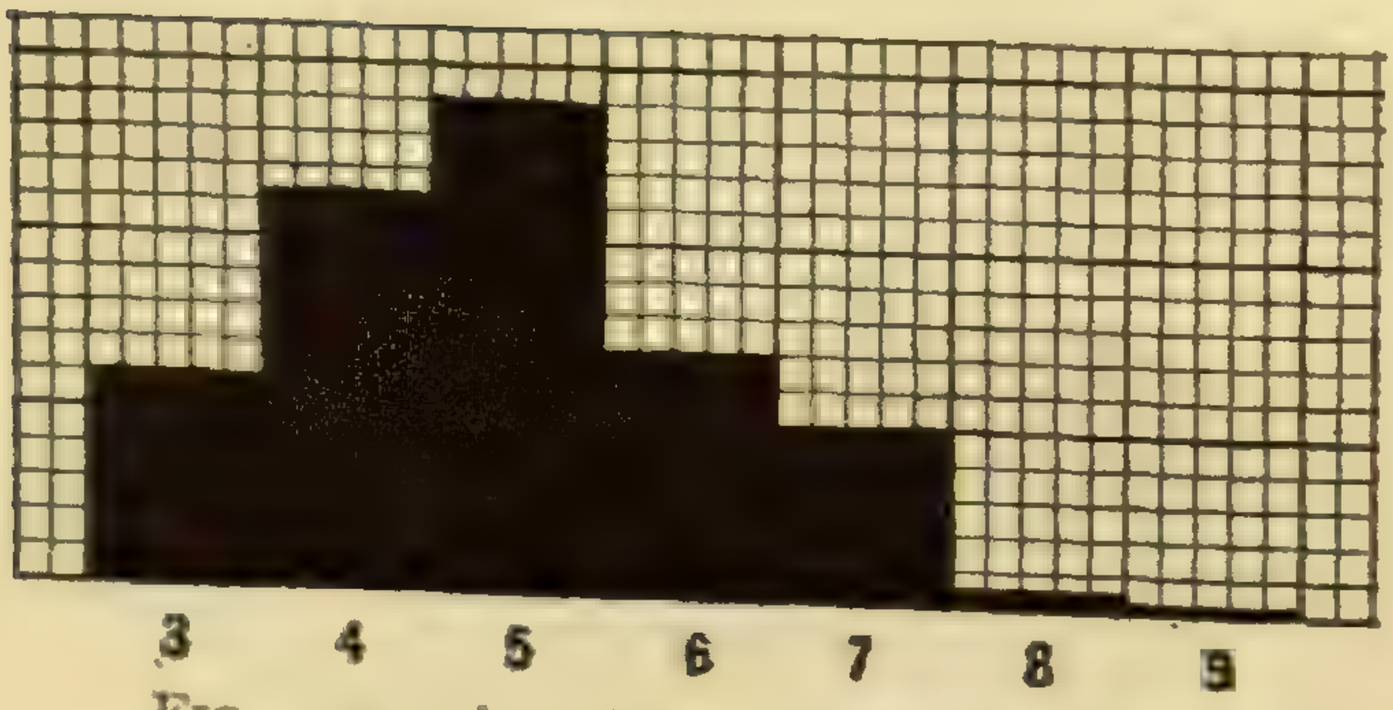

FIG. 29.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores from cow pea agar.

Cow pea agar.

$$M = 4.8657 \pm 0.0545$$
$$\sigma = 1.1885 \pm 0.0386$$
$$C.V. = 24.427 \pm 0.839$$
$$n = 214$$

It is seen that on these different media the mode varies materially, being low on pure agar, higher on CBA + sodium asparaginate, and still higher when glucose or starch is added. The mode is high also in natural media, such as pea agar and cow pea agar.

In the terms of the systematist, spores from pure agar measured 7.4–14.8 μ, mostly 11.1 μ; those from CBA+sodium asparaginate 7.4–25.9 μ, mostly 12.9 μ.

Septoria Lycopersici Speg. of Tomato

Grown on apple agar.

$M = 21.507 \pm 0.190$
$\sigma = 4.686 \pm 0.135$
C. V. $= 21.787 \pm 0.655$
$n = 278$

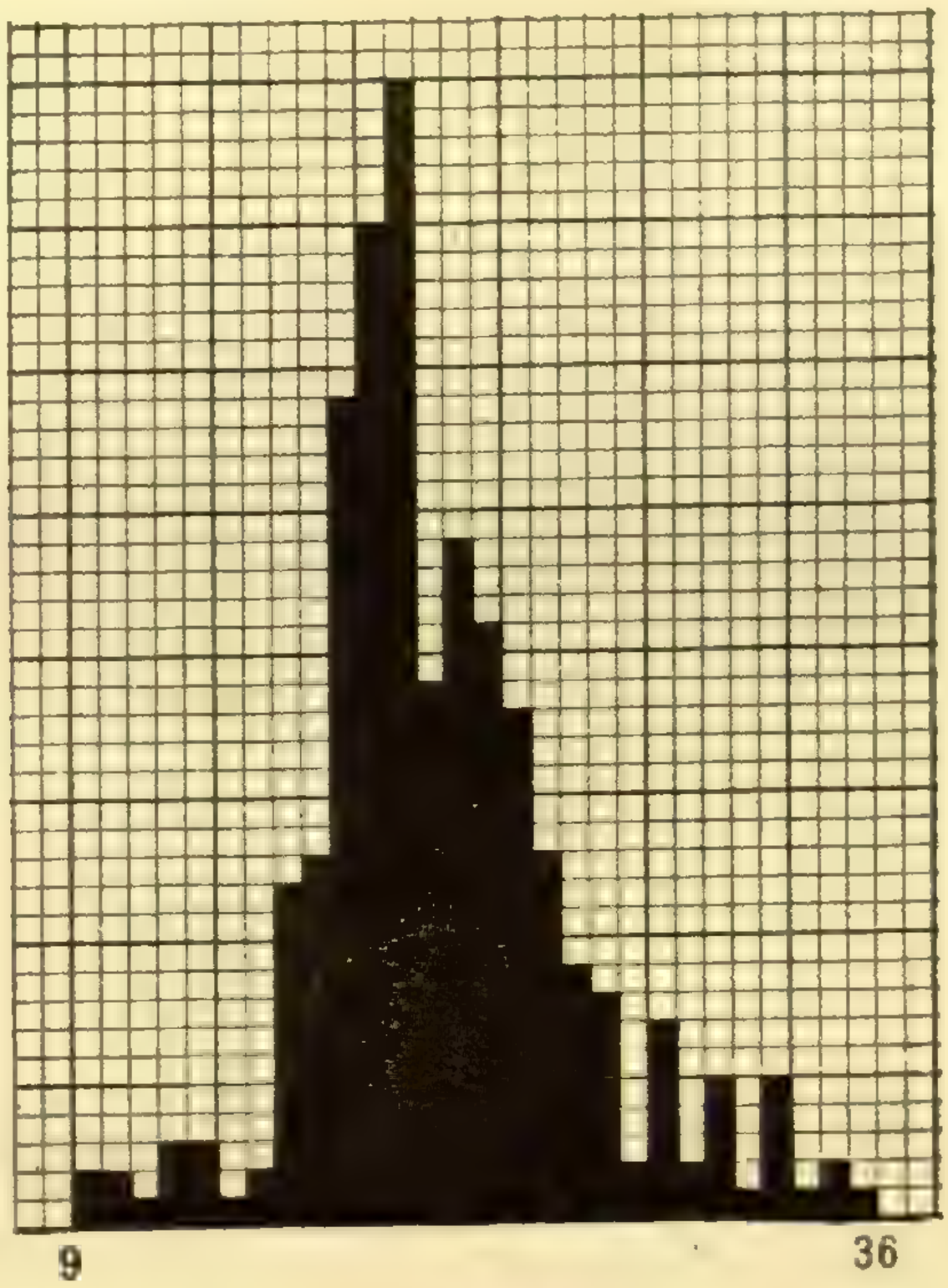

FIG. 30.—*Septoria Lycopersici* Speg. Polygon of spores on apple apar.

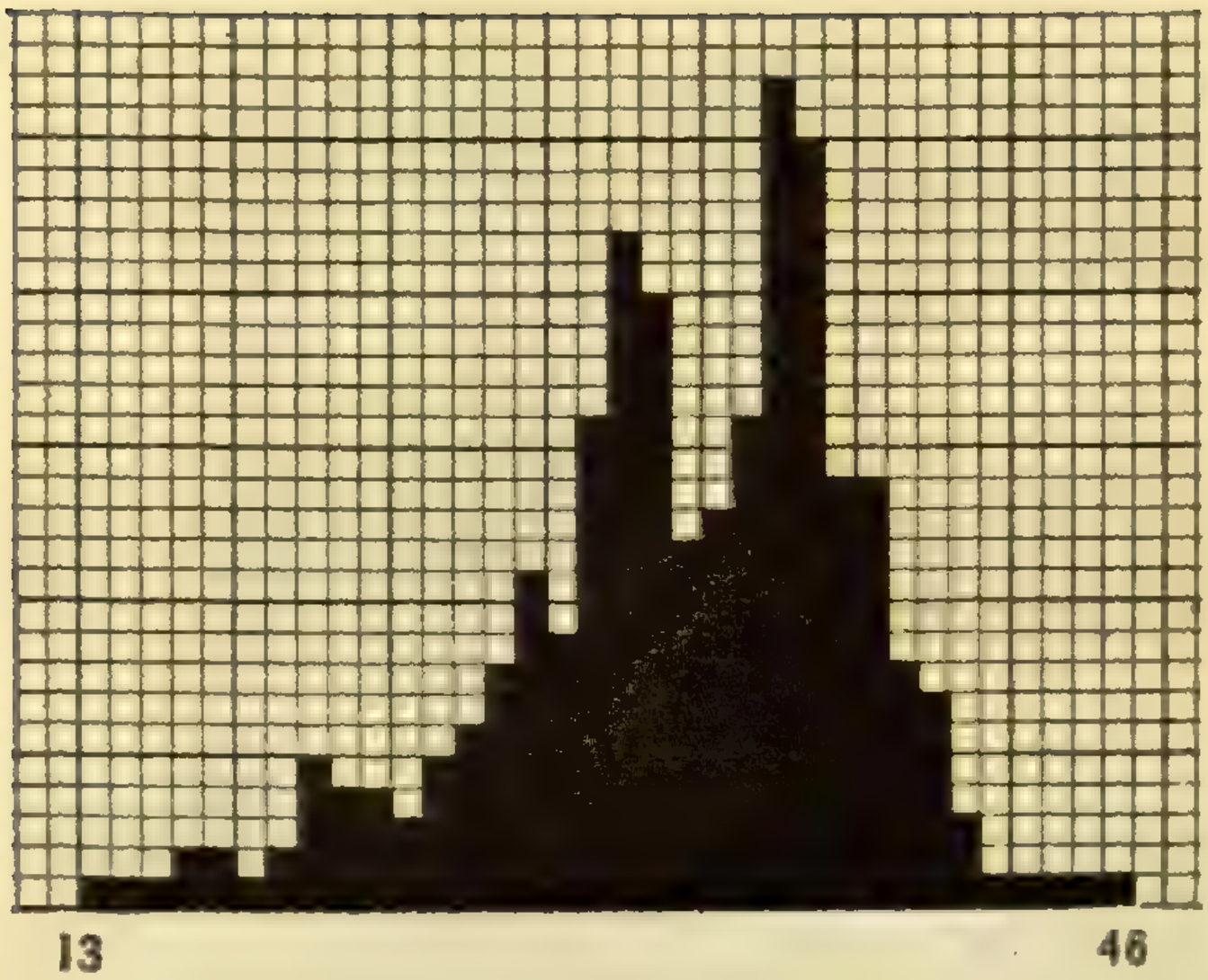

FIG. 31.—*Septoria Lycopersici* Speg. Polgyon of spores on pure agar.

Grown on pure agar.

$M = 31.675 \pm 0.242$
$\sigma = 5.879 \pm 0.171$
C. V. $= 18.560 \pm 0.559$
$n = 279$

Although the number of spores measured in the two last instances is not large, the fact of a tendency to larger spores on the poorer medium, apple agar, is evident. The spores measured 33.6–133.2 μ, mostly 81.4 μ; those on pure agar 48.1–181.3 μ, mostly 133.2 μ.

Ascospores of Sclerotinia Libertiana Fuckel

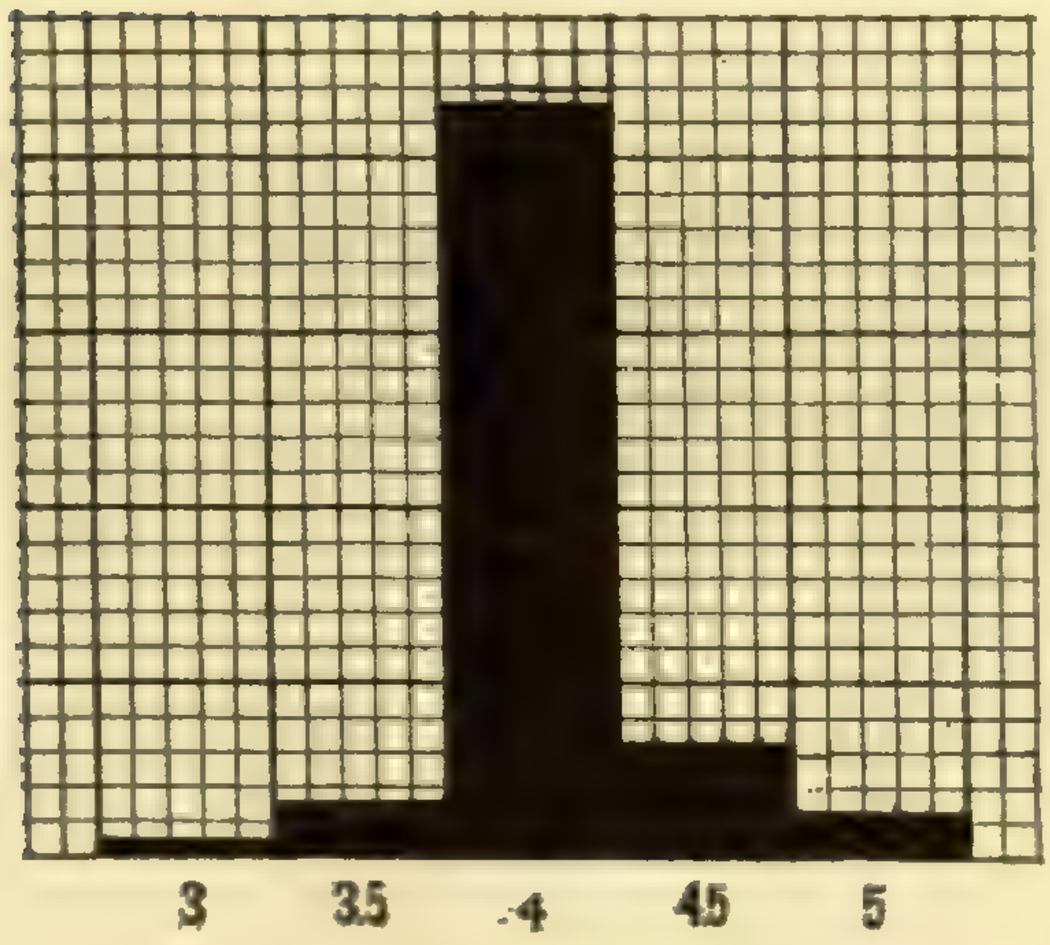

FIG. 32.—*Sclerotinia Libertiana* Fckl. Polygon of ascospores from middle-aged disk.

Spores were discharged spontaneously from the disk upon the cover glass, the disk being of middle age.

$$M = 4.0880 \pm 0.0166$$
$$\sigma = 0.2930 \pm 0.0117$$
$$C.\ V. = 7.168 \pm 0.290$$
$$n = 142$$

Spores were secured as in the last instance, but from very young disks.

$$M = 4.0393 \pm 0.0214$$
$$\sigma = 0.3743 \pm 0.0151$$
$$C.\ V. = 9.267 \pm 0.380$$
$$n = 165$$

No material difference in the size of the spores here appeared with the change in age of the disks.

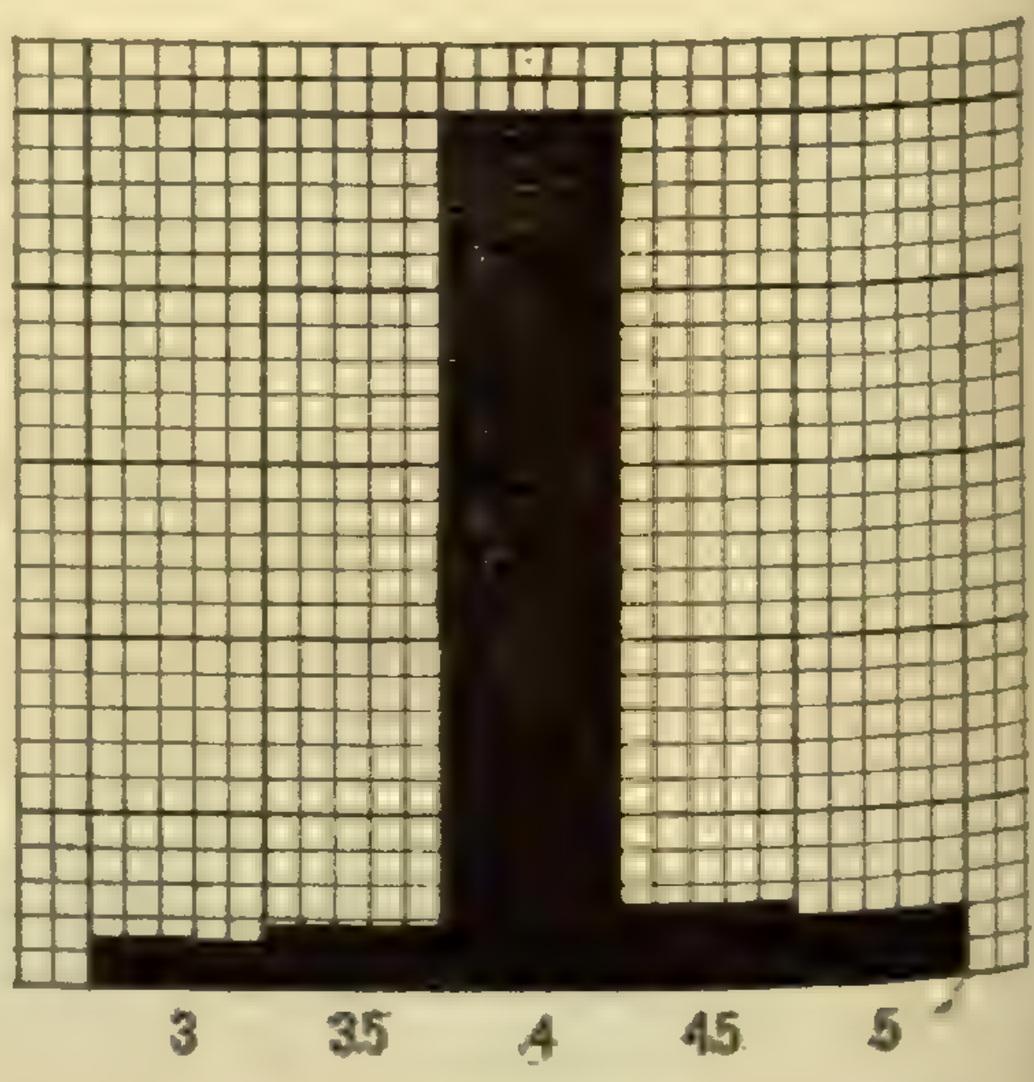

FIG. 33.—*Sclerotinia Libertiana* Fckl. Polygon of spores from young disk.

Diplodia macrospora Earle

Spores of this species, isolated from corn, were grown upon pea agar.

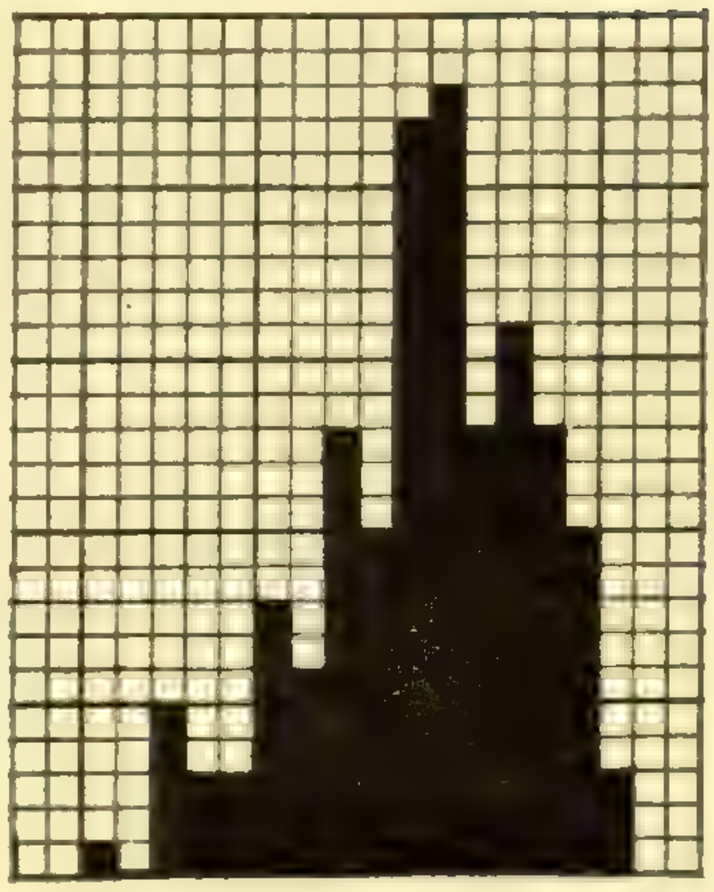

Fig. 34. — *Diplodia macrospora* Earle. Polygon of spores isolated from corn and grown upon pea agar; measurements showing length.

$M = 24.362 \pm 0.176$
$\sigma = 3.179 \pm 0.124$
C. V. $= 13.050 \pm 0.519$
$n = 149$

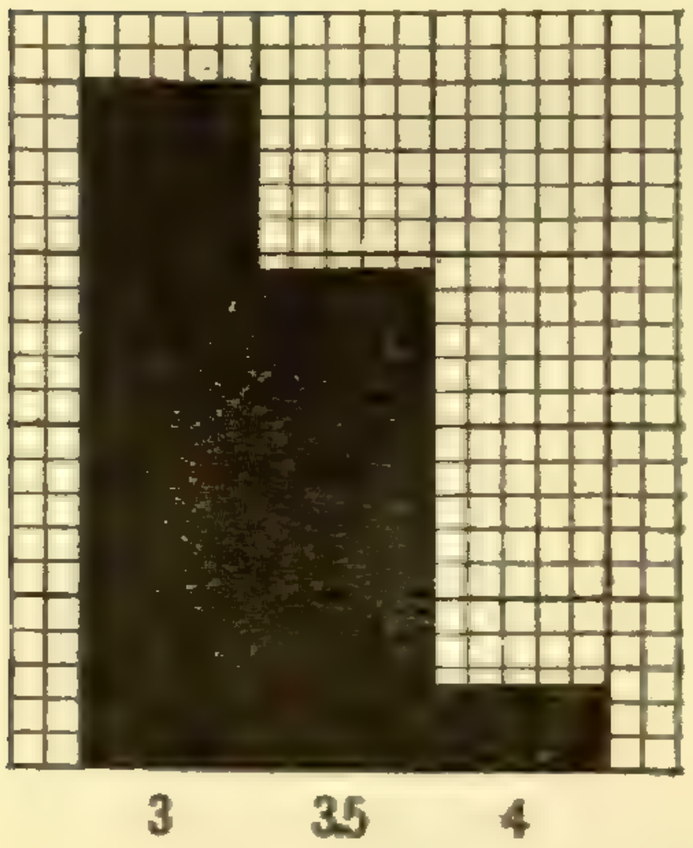

Fig. 35.—*Diplodia macrospora* Earle. Polygon of spores isolated from corn and grown upon pea agar; measurements showing thickness.

$M = 3.2595 \pm 0.136$
$\sigma = 0.2727 \pm 0.0096$
C. V. $= 8.367 \pm 0.297$
$n = 183$

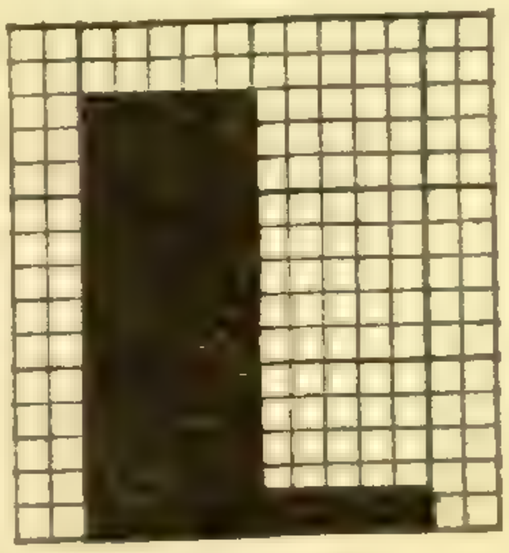

Fig. 36.—*Volutella fructi* S. & H. Polygon of spores showing width.

Volutella fructi S. & H.

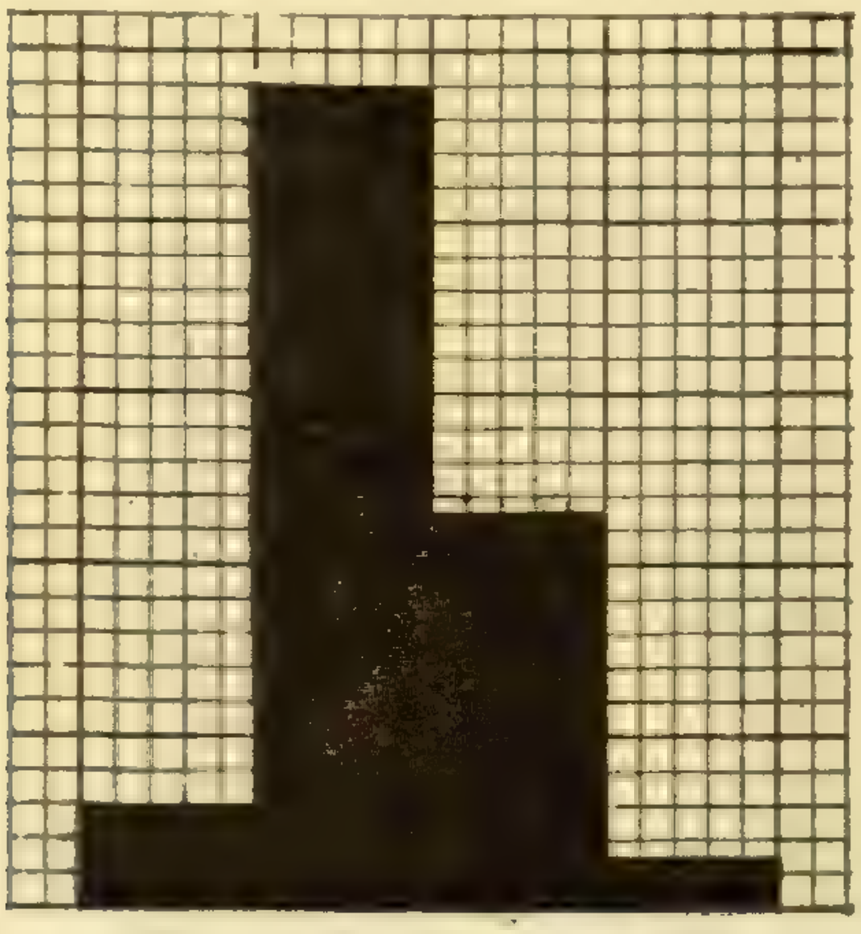

Fig. 37. — *Volutella fructi* S. & H. Polygon of spores showing length.

$M = 8.27 \pm 0.0276$
$\sigma = 0.5778 \pm 0.0195$
C. V. $= 6.986 \pm 0.237$
$n = 200$

The last five polygons are without particular significance and serve only to show the variation encountered in these forms.

General considerations

The bearing of these facts upon mycological taxonomy is apparent. If a fungus can be easily changed as regards its essential descriptive characters by a change in substratum, density of infection, or other environmental factor, these characters are worthless for descriptive purposes, unless the conditions under which they develop be accurately known.

There are two fundamental benefits from description: (1) to enable recognition of a particular form; (2) to aid in classification. The first of these is a necessary preliminary to the second, and it is with mere recognition that we have in many instances yet to deal in mycology, particularly among the group *Fungi imperfecti*, with its enormous genera, such as Septoria, Phyllosticta, and Cercospora, with their thousands of so-called species. While life-history work and infection experiments will do much, accurate recognition of the form in hand is a necessary preliminary even to this.

To reach any satisfactory basis, many fungi must be studied in culture, under suitable standard conditions, much after the fashion that bacteria are now studied.

NORTH CAROLINA AGRIC. EXPER. STATION AND COLLEGE
WEST RALEIGH

THE DEVELOPMENT OF THE GAMETOPHYTES AND FERTILIZATION IN *JUNIPERUS COMMUNIS* AND *JUNIPERUS VIRGINIANA*

ALICE M. OTTLEY

(WITH PLATES I—IV)

INTRODUCTION AND METHODS

The present paper is based upon a study of *Juniperus communis* and of *Juniperus virginiana*. *J. communis* was worked out more in detail, and throughout the following description it is the species referred to unless otherwise stated. Since this study was begun, two papers, SLUDSKY (**22**) and NORÉN (**20**), based on a study of *J. communis*, have appeared. In the main NORÉN'S observations have been confirmed by the present study. He gave a very complete history of the literature relating to Juniperus and it will not be necessary to repeat it here.

The pistillate and staminate cones of *J. communis* were collected about one and a half miles southeast of Wellesley, Mass., those of *J. virginiana* from the campus of Wellesley College and near-by places. The collection of the material began the second week in March, 1905, and continued until the latter part of June of the same year. Fresh material was gathered once a week from March 13 through the first week in May; from May 8 until June 26 collections were made twice a week. The material was kept in water and put up approximately every other day. Pistillate cones of *J. communis* for 1903, 1904, and 1905, and of *J. virginiana* for 1905 were collected; the staminate cones were put up until the shedding of the pollen, which in 1905 was on May 9 for *J. virginiana* and May 11 for *J. communis*. The fixing agent used was Flemming's chromacetosmic solution and the stain gentian violet and orange G.

Besides the slides prepared from this material I have had the privilege of studying one hundred slides which were prepared by Miss FERGUSON in the spring of 1903. These were of great value in giving the approximate dates at which to look for certain desirable stages,

as well as in showing the variation in time of the occurrence of certain phenomena due to varying climatic conditions of different seasons. It is interesting to note that the different stages of development as worked out by NORÉN from material collected in Sweden were, in general, from three to four weeks later than corresponding stages in the material collected at Wellesley.

MALE GAMETOPHYTE

Both *J. communis* and *J. virginiana* are dioecious, occasionally monoecious. RENNER (**21**) reported the presence of hermaphrodite flowers in *J. communis*. I searched for similar flowers during the period of collection, but was unable to find any. The microsporangia were fully developed and the microspore mother cells were already present in *J. virginiana* on March 28, but in *J. communis* the microsporangia were not differentiated until April 13. The sporophylls are cyclic in arrangement and are disposed in whorls of three, with the microsporangia on the under side next to the axis of the cone (*fig. 1*). On April 25 the cells of the archesporial tissue were undergoing their last division before the formation of the microspore mother cells. Following this division, each microsporangium consists of a central mass of polygonal microspore mother cells; surrounding these is a single layer of tapetal cells, rich in protoplasm and containing large nuclei, and a wall of two layers of cells (*fig. 2*), as has been described by other writers. The cells of the outer or superficial layer of the wall are large and tabular. They contain considerable resin, which causes them to stain diffusely.

Very soon after the differentiation of the microspore mother cells the heterotypic division begins. LOPRIORE (**18**) reported that the microspore mother cells of *Araucaria Bidwillii* also undergo a very short period of rest before this division is entered upon. As soon as the walls are formed about each microspore, the mother wall breaks down, setting the spores free in the sporangium.

The microspores increased in size and were shed May 11. The mature pollen grains of Juniperus do not differ as to content from the microspores. In the spring of 1903 pollination took place in *J. virginiana* on April 22, but in the spring of 1905 not until May 9. There is, then, a variation, due to seasonal or climatic conditions, of

more than two weeks in the time at which the pollen may be shed. The microspore in *J. communis* and in *J. virginiana* undergoes no division in the anther, and so far as I have been able to determine no prothallial cells are ever formed in them. This agrees with the condition found in *J. communis* (BELAJEFF **2**, NORÉN **20**), in Biota and Cupressus (STRASBURGER **27**), and in *Taxus baccata*, *J. sphaerica*, and *J. chinensis* (COKER **6**). In Thuja (LAND **14**), Libocedrus and Chamaecyparis (LAWSON **17**), a division takes place while the pollen grains are still in the anther and gives rise to the tube and antheridial cells, but in these also no prothallial cells are ever formed.

The mature pollen grain has two walls, a deeply staining outer one and a faintly staining inner one (*fig. 3*). The outer wall of the pollen grains appears slightly roughened in many instances. When pollination takes place, the one-celled pollen grain comes to rest on the nucellar cap, the cells of which immediately lose their nuclei and become more or less disintegrated and collapsed, forming a depression in the top of the nucellus (*fig. 6;* NORÉN, *pl. 1, fig. 5*). Fifteen days after pollination the microspore divided to form the tube and the antheridial cells. The antheridial cell is the smaller of the two and remains always at one end of the pollen grain, while the tube cell is larger, possesses less dense cytoplasm, and its nucleus occupies the center of the pollen grain. Many starch grains are present at this time in the tube cell. The nucleus is large, with a loose chromatic network, and contains one nucleolus (*fig. 5*). As described by NORÉN (**20**), immediately after the division to form the antheridial and the tube cells, the pollen grain germinates, and the tube pushes its way down into the tissue of the nucellus, but by June 26 it has reached only a short distance. The tube nucleus migrates into the tube, but does not reach the end of it until the following spring; whereas in *J. virginiana* the tube nucleus passes at once into the tip of the tube (*fig. 8*), and fertilization takes place in the early summer of the same year.

Soon after germination in *J. communis*, the exine is shed in the pollen chamber and remains there, a dark-blue shell, as has been described for Taxodium by COKER (**5**). In many cases the pollen grain end of the tube is raised up above the nucellar tip into the pollen chamber, giving a peculiar appearance. This may be caused by the

germination of the pollen grain before it reaches the nucellus, or it may be that the pollen tube finds difficulty in piercing the tissue of the nucellar cap, and in the effort to do so the pollen grain end is pushed up into the pollen chamber. From the appearance of the pollen tube lying in a horizontal plane on the nucellar cap before the tip enters the nucellus, it seems quite probable that the latter explanation is the more suitable one.

The following spring the antheridial cell divides to form two nearly similar cells, the stalk and the generative cells. The division was not observed, but on May 8 the two cells had been formed and were seen in the pollen grain portion of the male gametophyte (*figs. 9, 10*). NORÉN gives no figure illustrating this stage and speaks only of a stalk nucleus. The two cells soon move down the tube. At first they are very similar in appearance, but in a short time the generative cell is easily distinguished by its larger nucleus, and by the fact that it retains its own cytoplasm; while that of the stalk cell is not distinguishable after passing into the tube, as in Thuja (LAND **14**) (*figs. 9–12*). Not until after the stalk nucleus has passed the generative cell does the nucleus of the latter appear larger than either the stalk or the tube nucleus (*fig. 12*). At this time the generative cell increases rapidly in size, and its nucleus becomes several times larger than either of the other two nuclei. The difference in size of these nuclei is more marked in *J. communis* (*fig. 13*) than in *J. virginiana* (*figs. 11, 12*). Neither in *J. communis* nor in *J. virginiana* is it possible, as stated above, to detect the cytoplasm of the stalk cell in the pollen tube. That the entire cell loses its connection with the wall of the pollen grain and starts into the tube is clearly shown in *fig. 10*. But very early in its downward course its cytoplasm fuses with the general cytoplasm of the tube and can no longer be distinguished from it.

The end of the tube with its cytoplasmic contents advances toward the female prothallium and spreads over the upper end of the archegonial complex (*fig. 27*). The generative cell takes its position in the center of the tube near the tip, and strands of protoplasm extend from it to the lateral walls of the pollen tube and below to the tip. The stalk and the tube nuclei, which are usually of the same size and can no longer be distinguished from each other, are side by side below

the generative cell. This corresponds with the condition at this time in Thuja as described by LAND (**14**). As noted by NORÉN, no starch appears in the pollen tube at this time. Previous to the division of the generative cell its cytoplasm becomes decidedly alveolar in appearance (*fig. 13*), suggesting the appearance of this cell in Zamia (WEBER **29**), Cycas (IKENO **9**), and Sequoia (LAWSON **15**). NORÉN does not speak of this appearance, but describes the cytoplasm as coarse grained and containing a large amount of finely divided starch which might easily escape observation. I was unable to find any signs of starch in this cell at any time in its history.

Just before fertilization the generative cell divides and forms two cells equal in size (*fig. 27*), as shown and described by NORÉN. This agrees with Biota (STRASBURGER **27**), Thuja (LAND **14**), Taxodium (COKER **5**), and Cryptomeria and Sequoia (LAWSON **15, 16**). When mature the two sperm cells in Juniperus are hemispherical in shape and lie with their flat sides face to face, but not in contact, as described by NORÉN, and in Thuja by LAND (**14**). The division of the generative cell occurs normally in Juniperus after the tube has reached the neck of the archegonium, but in one instance two similar nearly spherical cells which resembled generative cells appeared in the pollen tube when it was about half-way through the nucellus.

It would seem that the two sperm cells may both be capable of functioning, as LAND (**14**) thinks is the case in Thuja. They are equal in size, the archegonia are borne in complexes, and the tip of the pollen tube is pressed against the neck cells of several archegonia, thus making it possible for the sperm cells from one tube to enter different archegonia. Two or three pollen tubes may reach the same archegonial complex, and there is very little branching of the tubes in their way through the nucellus.

FEMALE GAMETOPHYTE

On March 28 the ovuliferous cones had appeared, but there were no indications of ovules. The first stages in their development were observed May 1. There are, as a rule, three ovules borne in the same horizontal plane at the apex of the cone. The integument arises as a little swelling at the base of the nucellus, and by May 8 it had passed beyond the top of the nucellus. But one integument is present, and

it is free to the base of the nucellus (*fig. 14;* NORÉN, *pl. 1, fig. 5*). The arms of the integument are composed of three to five layers of cells. The micropyle is wide and deep, and no depressed pollen chamber is present until after pollination. The nucellar cap is composed of large clear cells, which in the upper layer project up, thus giving a jagged or irregular outline to the top of the cap. The cells beneath these are smaller, more nearly isodiametric, are arranged concentrically, and contain large darkly staining nuclei (*fig. 14*). NORÉN does not find this concentric arrangement until after pollination.

At the time of pollination, a drop of clear liquid resembling a crystal bead is deposited on the top of each of the three ovules. It is probable that the pollen falls on this drop and is drawn down into the micropyle by the drying of the liquid, as has been described for other gymnosperms. The closing of the micropyle is brought about by the rapid elongation of the inner row of cells of the arms of the integument. Occasionally the two arms meet in a straight line (*fig. 15*), as in Pinus (FERGUSON **7**). More often, however, the arms dovetail together (*fig. 16*), also figured by NORÉN. By this method the micropyle is even more securely closed. Following the elongation of the cells cross-walls are laid down, cutting the lengthened cells into smaller ones. COKER (**5**) describes a similar elongation of the inner row of cells in Taxodium, while Miss FERGUSON (**7**) finds it in the middle row of cells in Pinus, and LAWSON (**16**) states that in *Cryptomeria japonica* the subepidermal and the epidermal rows both elongate. NORÉN reports that the closing of the micropyle in *J. communis* has recently been described by KUBART (**13**), but I have not seen this paper.

Soon, in the lower part of the nucellus, several faintly staining cells appear, the so-called spongy tissue of STRASBURGER. It is one of these cells that, in the following spring, is differentiated into the macrospore mother cell. It is very probable that it was the presence of this spongy tissue which led HOFMEISTER (**8**) to the opinion that the macrospore mother cell developed in early summer. NORÉN says that usually the macrospore mother cell could be distinguished by the beginning of July, but that occasionally two or four cells enlarge, and then it is impossible to tell which is the macrospore mother cell until the follow-

ing spring. In my material, the macrospore mother cell in *J. communis* was never differentiated until the spring following pollination.

Soon after pollination, May 11 for *J. communis*, the ovules cease to grow and remain practically the same size until the following spring (*figs. 15, 16*). In *J. virginiana*, however, the ovules continue to grow, the macrospore mother cell is differentiated, the female gametophyte is formed, and fertilization takes place in the latter part of June or the first of July of the same year in which the ovules were pollinated. SLUDSKY (**22**) described fertilization in *J. communis* as taking place in the same year as pollination. Both NORÉN'S studies and the present investigation show that over a year elapses between pollination and fertilization in *J. communis*. It seems highly improbable that SLUDSKY could make a mistake in the age of the cones with which he was working, as suggested by CHAMBERLAIN (**3**) in a recent review in the BOTANICAL GAZETTE. Since the present study shows conclusively that the time elapsing between pollination and fertilization in different species of Juniperus may vary by nearly a whole year it seems far more probable that SLUDSKY was working with some other species than *J. communis*, and that in the species with which he worked fertilization does take place in the summer following pollination, as is the case in *J. virginiana*.

In *J. communis* growth began again in the early part of April. The macrospore mother cell appeared April 14 in the basal portion of the nucellus, and three days later the cell divided. In the prophase of the first division the cytoplasm is vacuolate except at one point, a short distance below the nucleus, where it is dense and stains more deeply (*fig. 18*), as noted by NORÉN. A similar densely staining mass of cytoplasm is present in all stages of division in the macrospore mother cell. No connection between this body and the division figures could be traced. COKER (**5, 6**) observed a similar condensation in Taxodium and in Thuja, but was unable to determine its significance. The presence of synapsis, the character of the spirem after synapsis, and the shape of the chromosomes indicate that this is a heterotypic division. In one slide studied there seemed to be evidence of a four-celled axial row, but from the material examined it was impossible to determine definitely whether the axial row consists of three or four cells. NORÉN states that there are three cells.

The basal cell of the axial row develops into the embryo sac. *Fig. 22* shows a two-celled embryo sac, with the disintegrating remains of the other cells of the axial row above it. Immediately after the first division of the macrospore nucleus, the two daughter nuclei take up a position at opposite poles of the embryo sac, and the cytoplasm becomes more vacuolate. The cells surrounding the developing prothallium are large, usually binucleate, and suggest a tapetum, as described for Taxodium by COKER (**5**). The nuclei of the prothallium divide simultaneously and form an ever-increasing ring of free cells (*fig. 23*). A layer of spongy tissue surrounds this ring and separates it from the other cells of the nucellus. By May 30 cell walls are laid down between the free nuclei, beginning at the periphery and extending toward the center. Contrary to SOKOLOWA'S (**23**) generalization for gymnosperms, and NORÉN'S observations for *J. communis*, cross-walls are formed before the central vacuole has entirely disappeared.

In the upper end of the prothallium a few cells do not divide by cross-walls, but remain long and narrow. These are the fundaments of the archegonia. By the latter part of May or first of June, the nuclei in these cells divide and give rise to the mother cell of the neck cells and to the central cell of the archegonium (*figs. 24, 25*). The central cell remains undivided until just before fertilization. Its nucleus is in the upper half of the archegonium, and below it is a large cylindrical vacuole. The cytoplasm stains faintly, and the nucleus contains several nucleoli. As a result of the division of the central cell, there arise the egg cell and the ventral canal nucleus. As observed by NORÉN (**20**) and SLUDSKY (**22**) no distinct ventral canal cell is ever formed. During the division the cytoplasm stains very deeply and presents a most peculiar appearance. Scattered throughout the entire cytoplasm are many bodies with deeply staining centers which are the so-called protein vacuoles. Three centers of radiations are present in the cytoplasm at this time, two below the large vacuole and one above it. The radiations appear to have no connection with the division of the central cell, but they are never seen so clearly at any other time as during this mitosis. Somewhat similar radiations are described by COKER (**5**) for Taxodium. NORÉN (**19, 20**) and SLUDSKY (**22**) figure and describe them for Juniperus,

but cannot explain them. *Fig. 26* shows these radiations in an early prophase of the division of the central cell.

After the ventral canal nucleus is cut off, it moves to the micropylar end of the cytoplasm and usually disorganizes before the pollen tube bursts. *Fig. 28* shows an unusually large ventral canal nucleus. The egg nucleus increases in size and moves slowly toward the central vacuole, taking up a position just above it. It usually possesses a beautiful chromatin network and a large nucleolus or several smaller nucleoli. At this time both the radiations and the protein vacuoles disappear, and many darkly staining specks appear in the egg cytoplasm (*fig. 27*). Contrary to what I have observed and SLUDSKY (**22**) has reported, NORÉN (**20**) says that these centers of radiations, so noticeable at the time of the division of the central cell, increase in size during the maturation of the egg. There is no evidence whatsoever of this in the material which formed the basis of the present study.

The archegonia form a complex which is surrounded by a layer of sheath cells. These are small and filled with deeply staining protoplasm. HOFMEISTER (**8**) says that Juniperus frequently has archegonia in abnormal positions and NORÉN has made the same observation. The only case of archegonia in abnormal positions that was observed, is shown in *fig. 36*. Here the archegonium is outside of the layer of sheath cells, but in other respects it appears normal.

FERTILIZATION

Fertilization follows directly upon the division of the generative cell and the maturation of the egg cell. In the summer of 1905 fertilization in *J. communis* occurred on June 17, 20, and 21. In ovules put up on the same day were found divisions of the central cell to form the egg cell and ventral canal nucleus, undivided generative cells, sperm cells, fertilization, and proembryos. This would indicate that these divisions take place very rapidly. A mass of densely staining, coarse-grained cytoplasm accompanies the sperm nucleus on its entrance into the egg. Whether the other contents of the tube enter the archegonium was not clearly determined; in one preparation there were densely staining bodies in the upper part of the archegonium, which might be the disorganized remains of the

other nuclei. NORÉN states that as a rule the two free nuclei do not enter the archegonium. The sperm cell loses its cytoplasm and passes directly to the egg nucleus. When the two nuclei have come in contact, they appear of about the same density and each contains a nucleolus and several secondary nucleoli (*fig. 29*). The only apparent difference is in size, the sperm nucleus being somewhat smaller. In one instance they were almost equal in size, and a dense mass of cytoplasm almost completely surrounded them.

This densely staining mass is doubtless the cytoplasm of the sperm cell (*fig. 30*). SLUDSKY (**22**) describes the nucleus of the functional sperm cell as escaping from its cytoplasm and fusing with the egg nucleus, while the rest of the sperm cell forms a cap over the fusion nucleus. Whether the two sexual nuclei fuse and form one nucleus could not be determined with absolute certainty. In one preparation a nucleus was present a little below the middle of the archegonium, with a small vacuole above it and one below it. The nucleus is at rest, a chromatic network is present, and a large nucleolus (*fig. 31*). It seems very probable that this is a fusion nucleus on its way to the bottom of the archegonium, as this is not the position which the egg nucleus ordinarily has before fertilization, and no other nucleus was present in this archegonium.

SLUDSKY (**22**) observed a large vacuole in the upper part of the archegonium after fertilization. He thinks this originates by the flowing together of the many small vacuoles, which he believes enter the archegonium from the pollen tube. It seems to me, however, that this vacuole is but the remains of the large vacuole present before fertilization. NORÉN (**19**) finds that the large central vacuole of the egg often disappears before the conjugation of the sexual nuclei.

In one case there occurred what appeared to be the fusion of the ventral canal nucleus with the second sperm nucleus. Two nuclei are present in the chalazal end of this archegonium. These are unquestionably the first two cells of the proembryo. Thus the fusing nuclei in the micropylar end of the archegonium cannot be the egg and the sperm nucleus (*fig. 35*). LAND (**14**) has reported a similar instance in Thuja and considers it a case of the fertilization of both the ventral canal nucleus and of the egg nucleus in the same archegonium.

PROEMBRYO AND EMBRYO

I cannot confirm STRASBURGER'S (**24**) observation that the fusion nucleus moves to the organic apex of the archegonium before it divides. *Fig. 32* shows two nuclei in the resting stage and surrounded with cytoplasm of netlike appearance, about half-way between the middle of the archegonium and its lower extremity. While the cells of the proembryo are undergoing division, the cytoplasm is alveolar and very similar to that in the generative cell just before fertilization, except that the network is slightly finer (*fig. 34*).

Following fertilization, the three sporophylls of the pistillate cone fuse over the three ovules and form a berry-like fruit. As a rule the mature fruit contains but one or two seeds, the other ovules or ovule having ceased to grow before reaching maturity.

PHYLOGENY

From this study of Juniperus, it will be seen that in many respects this genus seems to be of more modern origin than many of the other gymnosperms. The cyclic arrangement of leaves and sporophylls, the absence of prothallial cells in the mature pollen grain, the absence of a ventral canal cell, and the presence of two functional sperm cells over an archegonial complex lead to this conclusion. JEFFREY (**10, 11**), in his investigations on the woods of the different gymnosperms, says that the wood of Juniperus and other Cupresseae indicates that they belong to a family more modern than the Abieteae. And THOMSON (**28**), as a result of his study of the development of the megaspore coat and of the tapetum, concludes that "the Abieteae are the most ancient group of the Coniferales and the Taxeae the most recent, that the Taxoideae and Podocarpeae are complex, including both ancient and recent forms; and that the Cupresseae occupy an intermediate position in the phylogenetic series."

SUMMARY

The staminate cones consist of many sporophylls, each bearing microsporangia on the under side near the axis.

The pistillate cones contain three ovules, each subtended by a sporophyll.

The mature pollen grain contains but one cell, no prothallial cells being present.

The macrospore mother cell first appears in *J. communis* one year after pollination. The first mitosis taking place within it shows clearly the stages characteristic of heterotypic division.

The contents of the pollen grain divide into antheridial cell and tube cell soon after pollination.

In *J. communis* the antheridial cell remains in the pollen grain and does not divide until April of the next spring.

In *J. virginiana* the antheridial cell divides in the early summer of the same year in which pollination takes place, and fertilization occurs in the latter part of June or first of July.

The generative cell and the stalk cell migrate immediately into the tube.

The stalk nucleus passes the generative cell and lies near the tube nucleus.

The generative cell does not divide until after the pollen tube has reached the archegonia and just before fertilization.

Two similar sperm cells are formed, each hemispherical in shape.

The female prothallium develops, as in the other gymnosperms, by free cell formation in a hollow sphere of cytoplasm. After many nuclei are formed, cell walls are laid down between them. These cells grow toward the center, but before they meet, cross-walls are laid down.

The archegonia develop from superficial cells at the micropylar end of the prothallium. They are arranged in a group, and the entire complex is surrounded by a single layer of sheath cells.

During the same day that the generative cell divides, and presumably before its division, the central cell of the archegonium divides.

A ventral canal nucleus is formed and remains throughout its life free in the cytoplasm of the egg, that is, no true ventral canal cell is ever cut off. This nucleus remains at the tip of the egg and, as a rule, soon disintegrates.

After fertilization the first division occurs before the fusion nucleus has passed to the organic apex of the archegonium.

In conclusion I wish to express my sincere gratitude to Professor

MARGARET C. FERGUSON, under whose direction the present investigation was carried on, and to whom I am greatly indebted for suggestions and encouragement.

WELLESLEY COLLEGE

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EXPLANATION OF PLATES I—IV

The figures were drawn with the aid of an Abbé camera lucida. The degree of magnification is indicated in the description of each figure. The following abbreviations are used in connection with the figures. Antheridial cell, *a;* archegonium, *arch;* archesporial tissue, *a. t;* central cell, *c;* central nucleus, *c. n;* cytoplasmic condensation, *c. c;* egg nucleus, *e. n;* generative cell, *g;* inner wall, *i. w;* integument, *i;* micropyle, *M;* microspore mother cell, *Mi. m. c;* microsporangium, *Mg;* microsporophyll, *Mp;* nucellar cap, *n. c;* neck cells, *nk;* nucellus, *nuc;* outer wall, *o. w;* pollen chamber, *p. c;* prothallium, *pro;* protein vacuole, *p. v;* starch, *s;* secondary nucleoli, *s. nu;* sperm cell, *sp;* sperm nucleus, *s. n;* spongy tissue, *s. t;* sheath cell, *sh. c;* stalk cell, *st. c;* stalk nucleus, *st. n;* tapetum, *t;* tube cell, *t. c;* tube nucleus, *t. n;* ventral canal nucleus, *v. c. n.*

PLATE I

FIG. 1.—Longitudinal section of staminate cone of *J. communis;* April 25, 1905. ×62.

FIG. 2.—Microsporangium of *J. communis*, showing microspore mother cells, tapetum, and wall; May 1, 1905. ×382.

FIG. 3.—Mature microspore of *J. communis*, just before pollination. ×1850.

FIG. 4.—Pollen grain of *J. communis*, from the nucellus of an ovule, in prophase of first division; May 26, 1905. ×1850.

FIG. 5.—A two-celled pollen grain of *J. virginiana*, two weeks after pollination. ×1850.

FIG. 6.—Nucellar cap of *J. virginiana*, showing disintegration of cells beneath the pollen grain. ×116.

FIG. 7.—Early stage in the piercing of the nucellar cap by the pollen tube of *J. communis;* June 3, 1905. ×884.

FIG. 8.—Young pollen tube of *J. virginiana;* June 1, 1905. ×382.

FIG. 9.—Upper part of the pollen tube of *J. communis*, showing the stalk cell and the generative cell; May 8, 1905. ×1850.

FIG. 10.—Pollen tube of *J. communis*, showing the tearing-away of the cytoplasm of the stalk cell from the wall of the old pollen grain; May 10, 1905. ×884.

FIG. 11.—Portion of pollen tube of *J. virginiana*, midway through the nucellus, showing the generative cell with the tube and the stalk nuclei just beneath it. ×1850.

PLATE II

FIG. 12.—Lower portion of an older pollen tube of *J. virginiana*, showing the two free nuclei below the generative cell. ×1850.

FIG. 13.—The generative cell of *J. communis*, just before division; June 9, 1903. ×884.

FIG. 14.—Longitudinal section of ovule of *J. communis*, showing nucellus and integument; spongy tissue not yet clearly differentiated; May 19, 1905. ×116.

FIG. 15.—Longitudinal section of ovule of *J. virginiana*, showing an occasional method of closing the micropyle. ×116.

FIG. 16.—Longitudinal section of ovule of *J. communis* in winter condition, showing usual method of closing of micropyle; March 28, 1905. ×116.

FIG. 17.—Macrospore mother cell of *J. communis;* April 14, 1905. ×704.

FIG. 18.—Macrospore mother cell of *J. communis*, in prophase of division with spongy tissue around it; April 17, 1905. ×704.

FIGS. 19–21.—Different stages in the division of the macrospore mother cell of *J. communis;* April 17, 1905. ×1850.

FIG. 22.—Two-celled embryo sac of *J. communis;* May 1, 1905. ×704.

PLATE III

FIG. 23.—Longitudinal section of the nucellus of *J. communis*, showing the hollow prothallium almost a year after pollination; May 5, 1905. ×188.

FIG. 24.—Prothallium of *J. communis*, almost closed at the center, with archegonia at the micropylar end; May 30, 1903. ×62.

FIG. 25.—Stages in the development of archegonia in the same archegonial complex of *J. communis;* May 30, 1903. ×704.

FIG. 26.—Longitudinal section of archegonium of *J. communis*, at the time of the division of the central cell; nucleus in prophase of division; large central vacuole and prominent radiations; June 20, 1905. ×704.

FIG. 27.—Longitudinal section of an archegonial complex of *J. communis*, with the tip of the pollen tube above it; June 20, 1905. ×382.

PLATE IV

FIG. 28.—Upper portion of archegonium of *J. communis* just before fertilization; June 20, 1905. ×1016.

FIGS. 29, 30.—Conjugation of sexual nuclei of *J. communis*, showing sheath of denser cytoplasm; June 20, 1905. ×1850, 884.

FIG. 31.—Fusion nucleus of *J. communis* between two vacuoles; June 20, 1905. ×884.

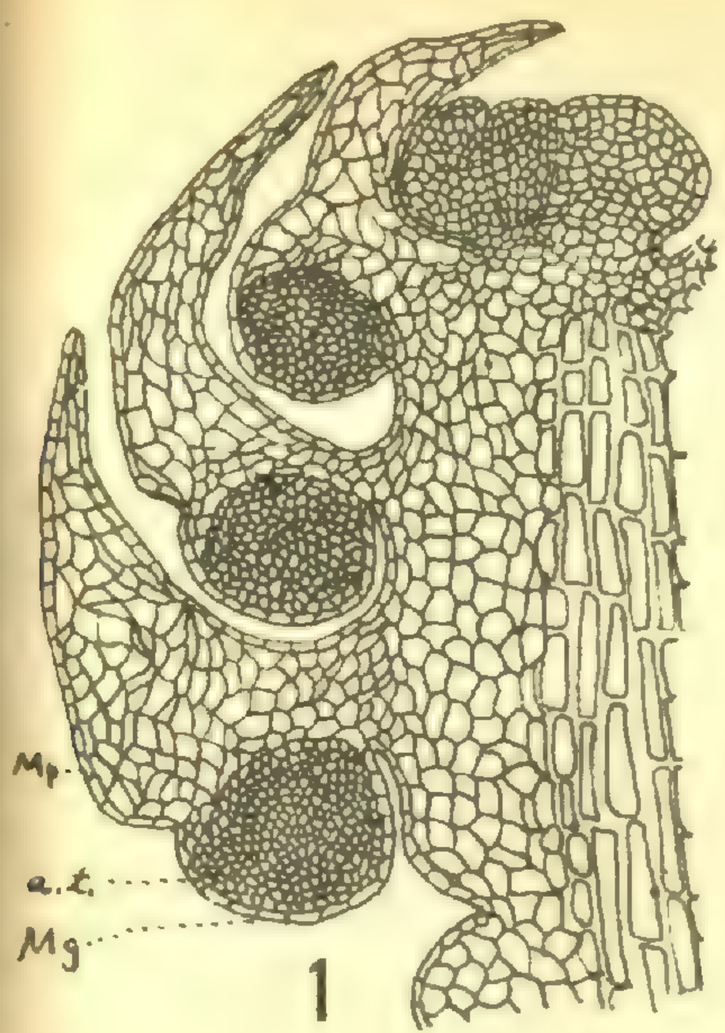
FIG. 32.—Two-celled proembryo of *J. communis*, surrounded by mass of nutritive substance; June 9, 1903. ×382.

FIG. 33.—Second division after fertilization at base of archegonium of *J. communis;* June 17, 1905. ×884.

FIG. 34.—Divisions to form the eight-celled proembryo of *J. communis;* June 20, 1905. ×884.

FIG. 35.—Probable fertilization of the ventral canal nucleus of *J. communis;* June 17, 1905. ×160.

FIG. 36.—Abnormal position of an archegonium of *J. communis*, just outside the complex; June 20, 1905. ×382.



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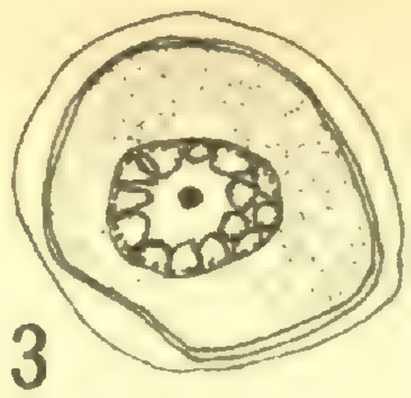

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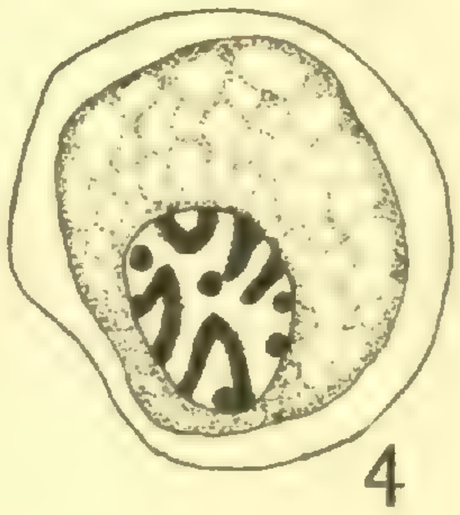

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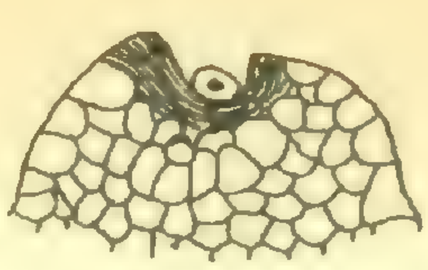

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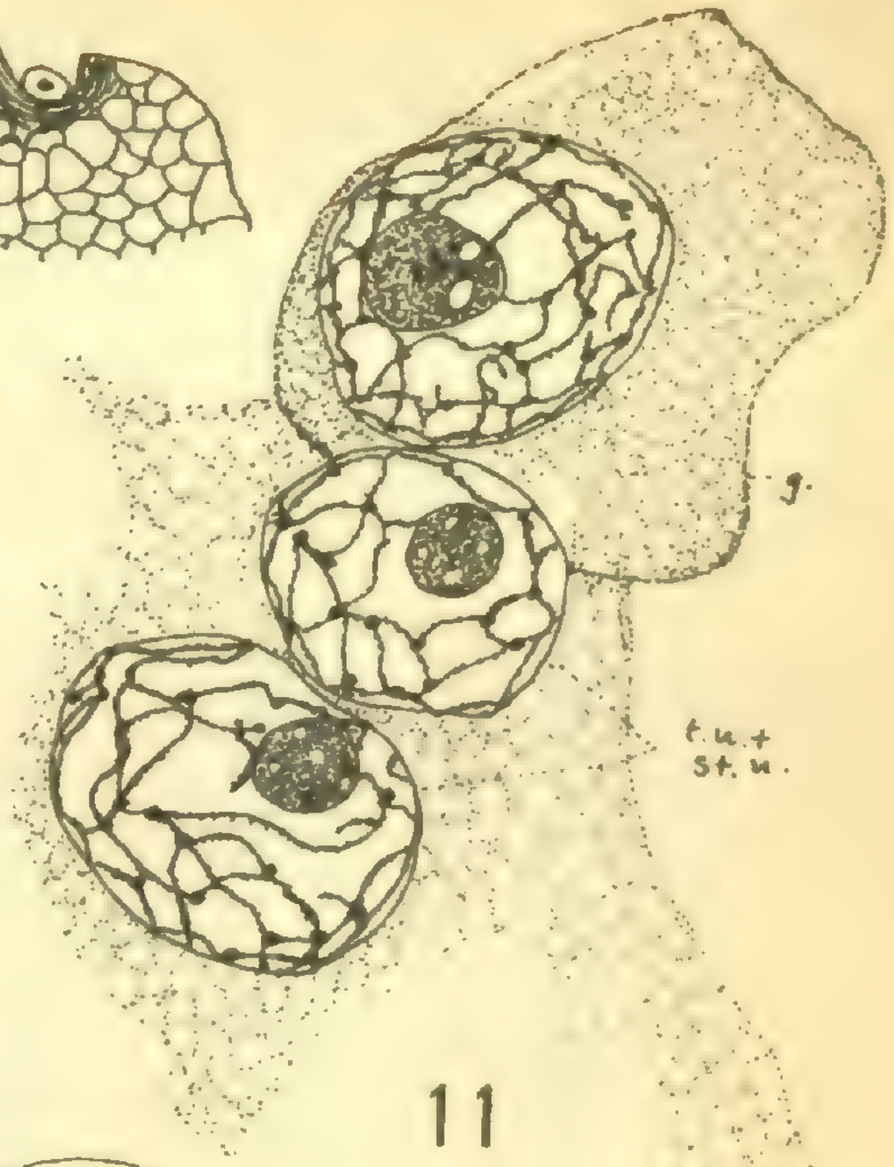

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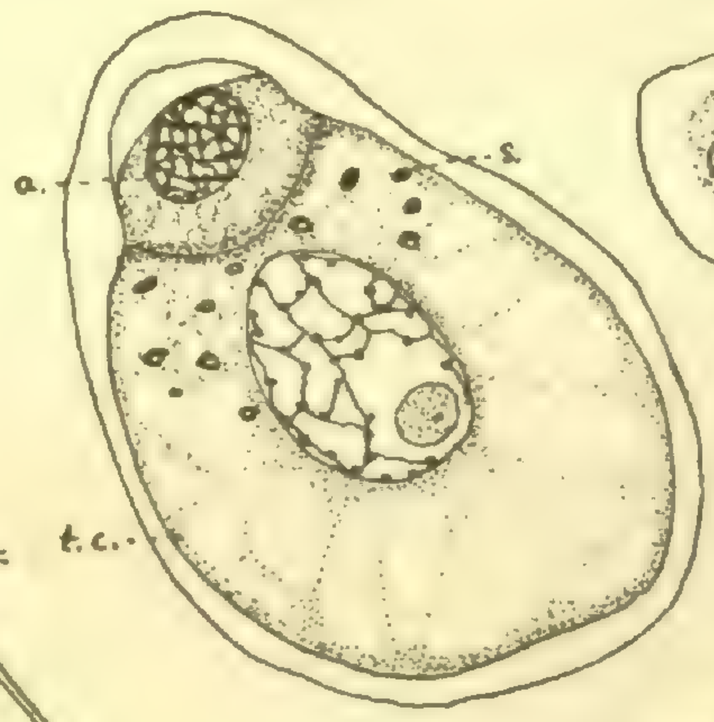

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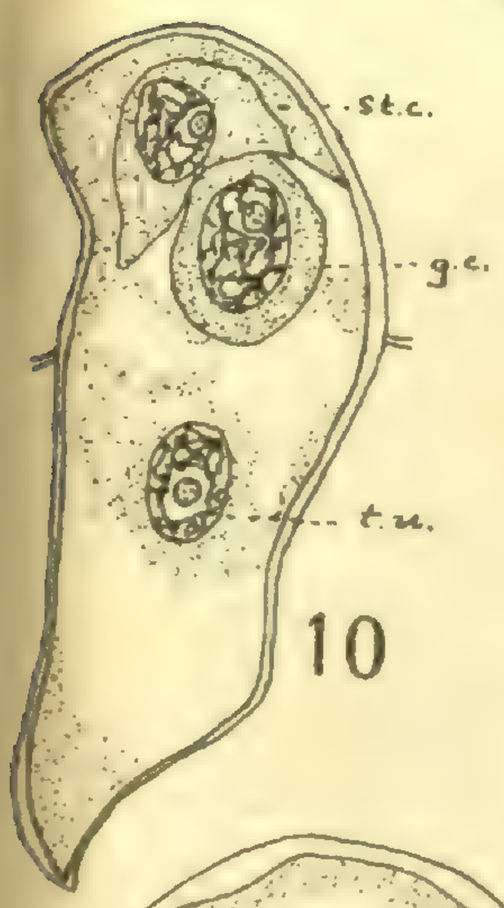

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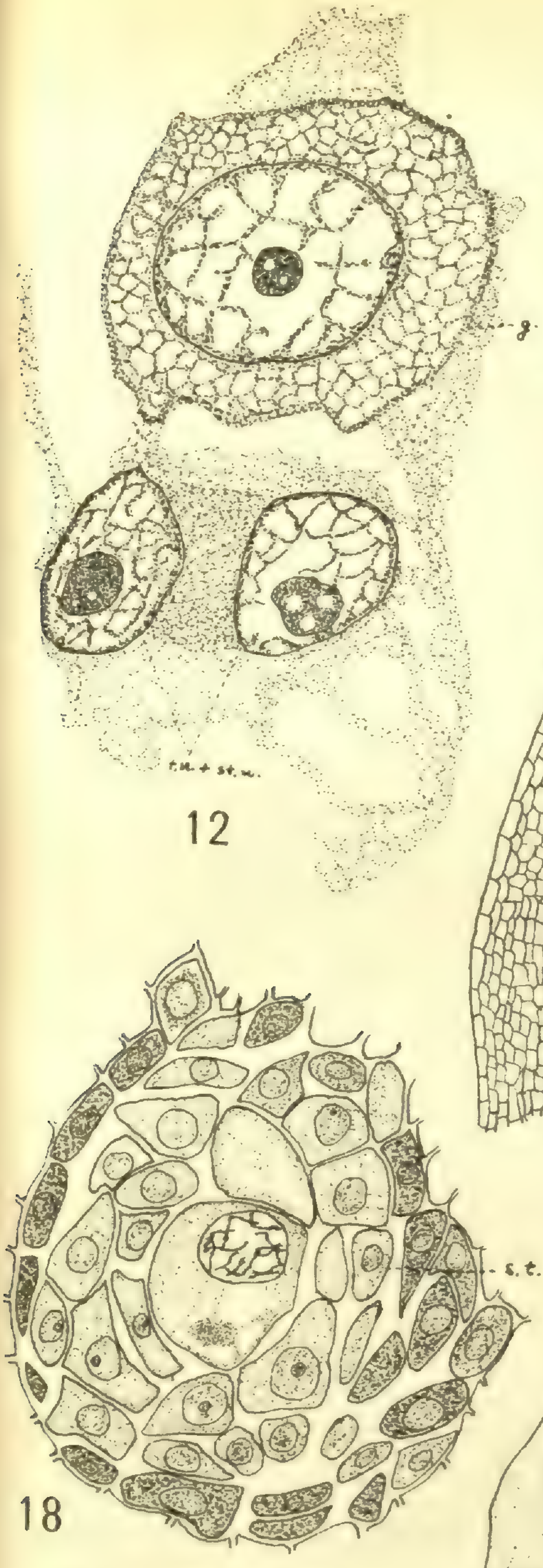

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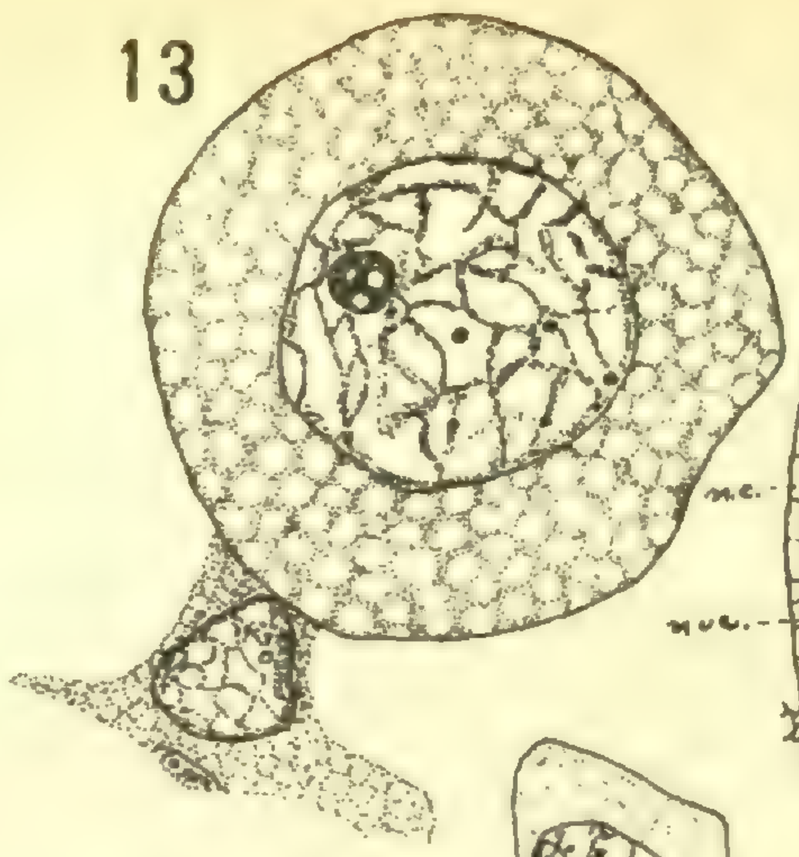

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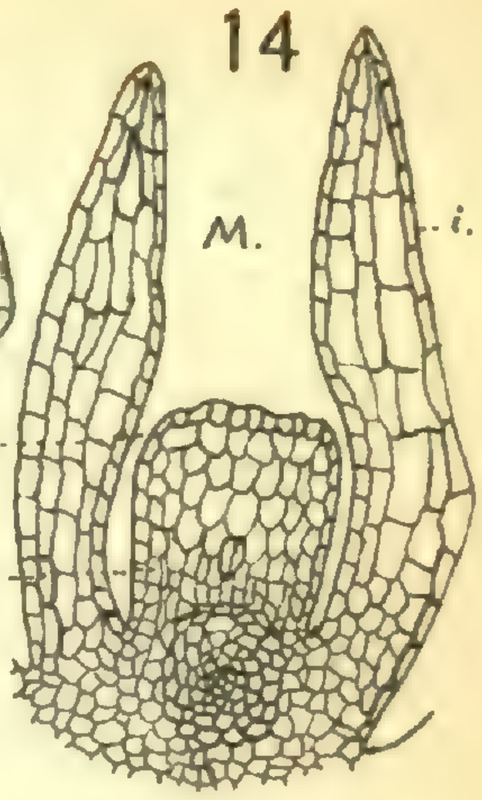

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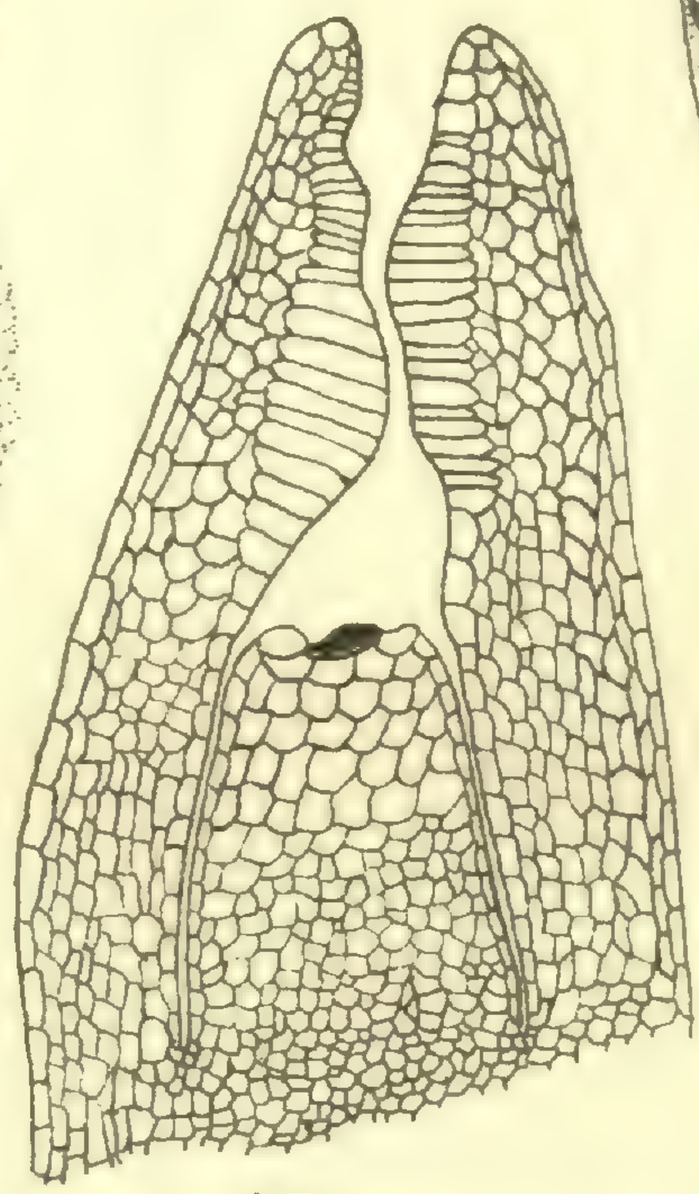

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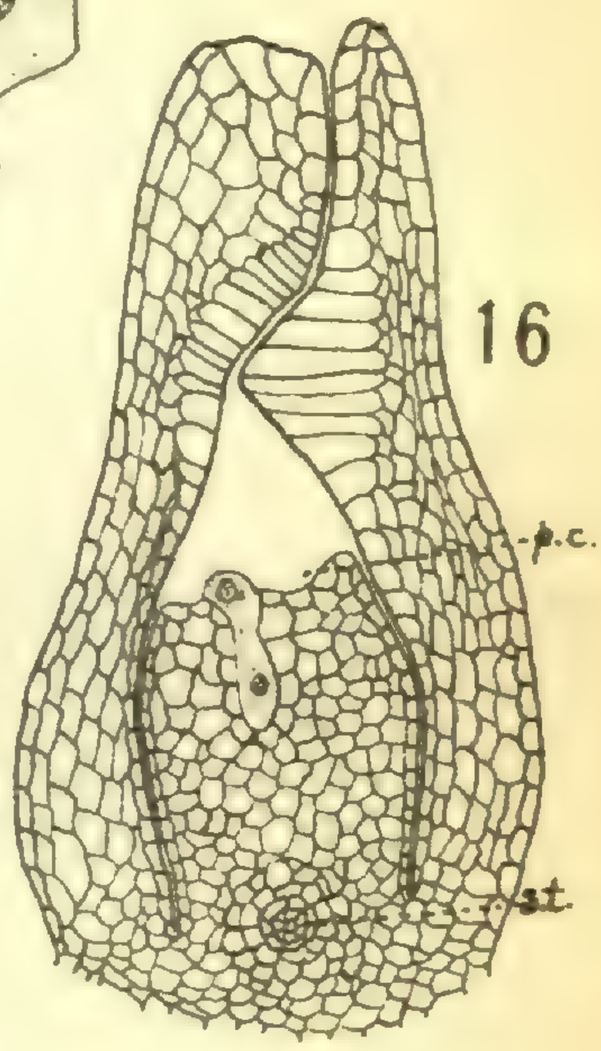

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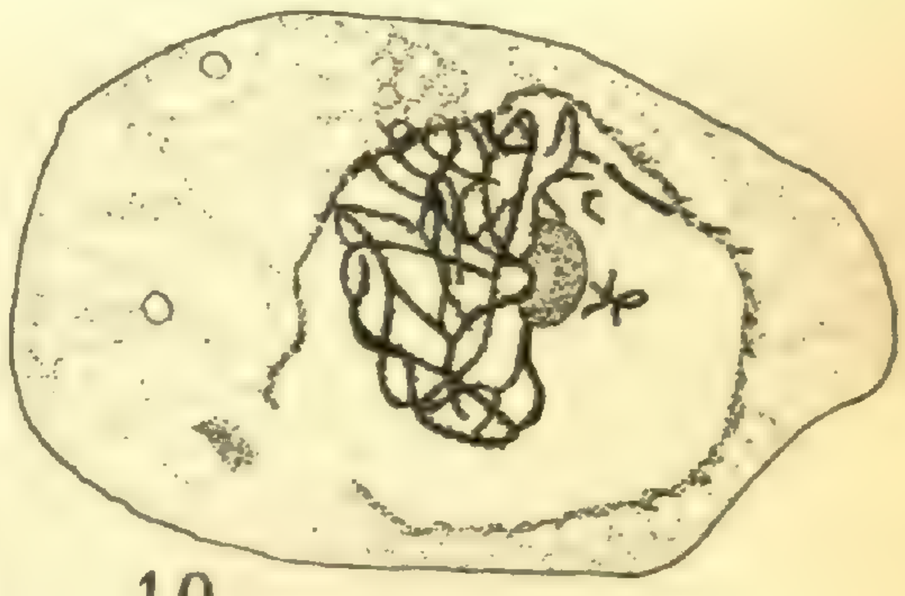

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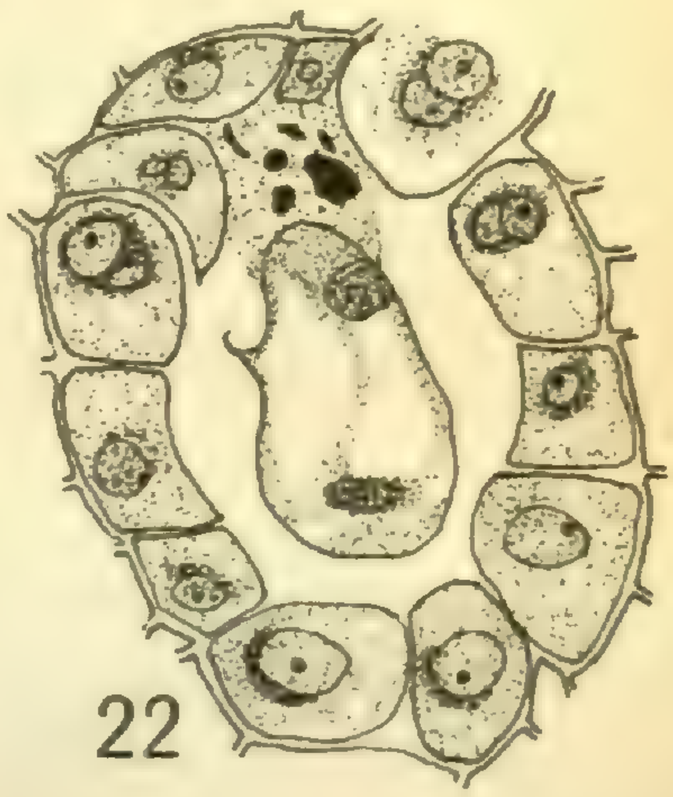

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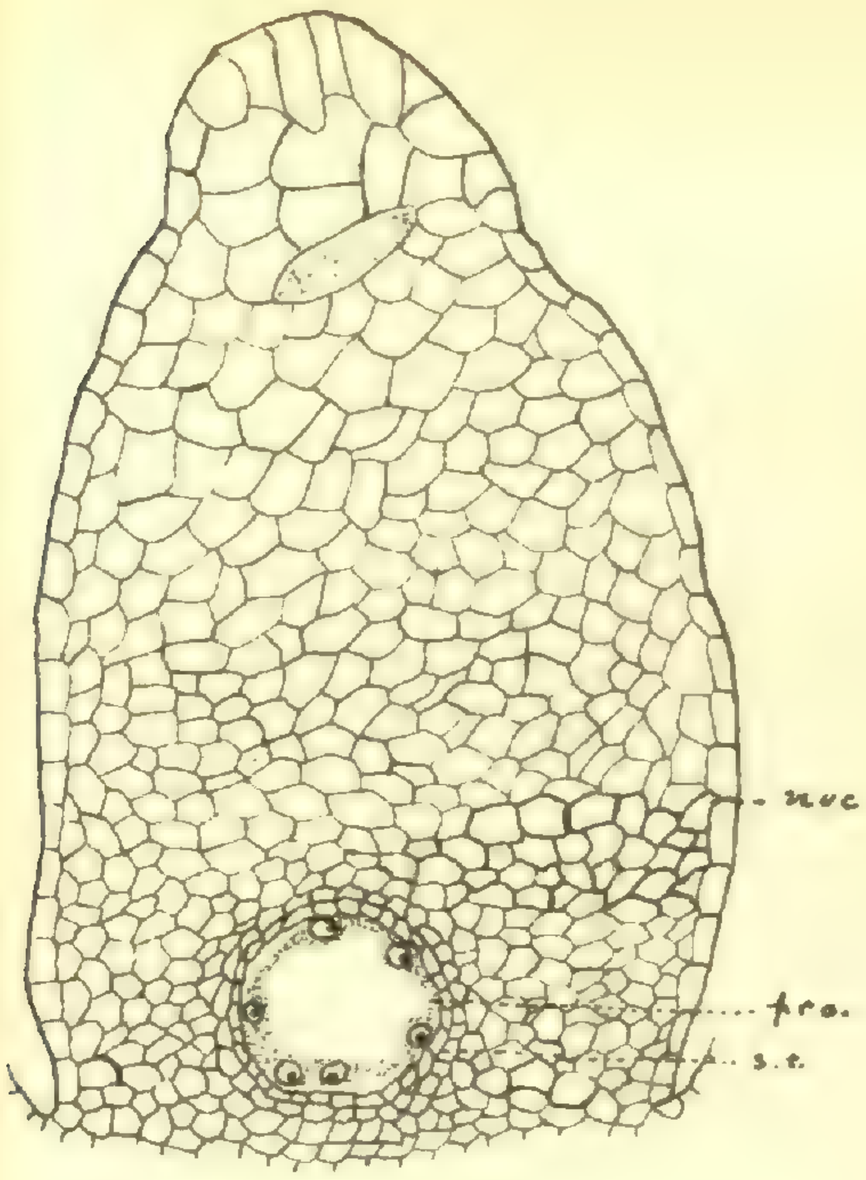

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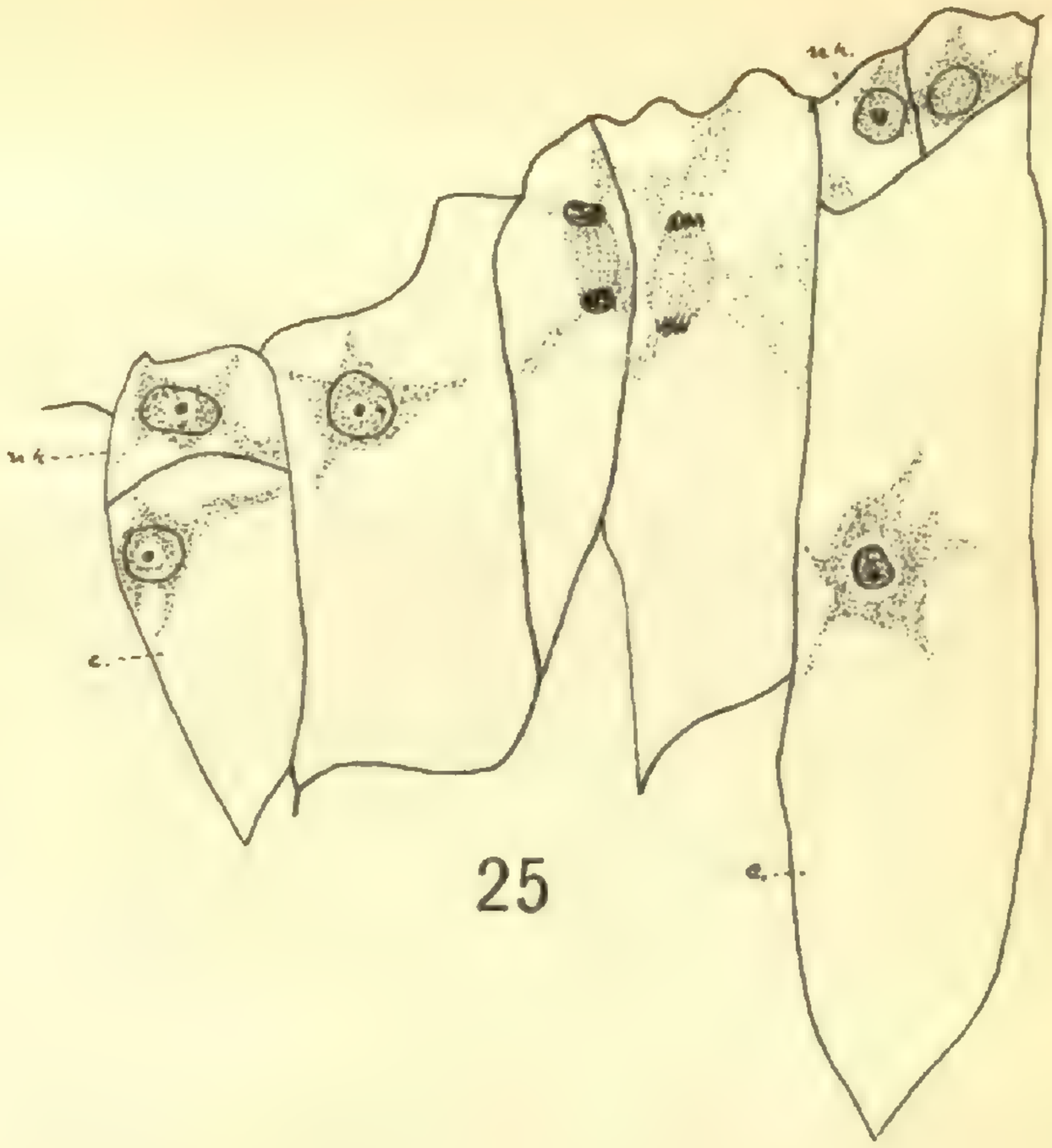

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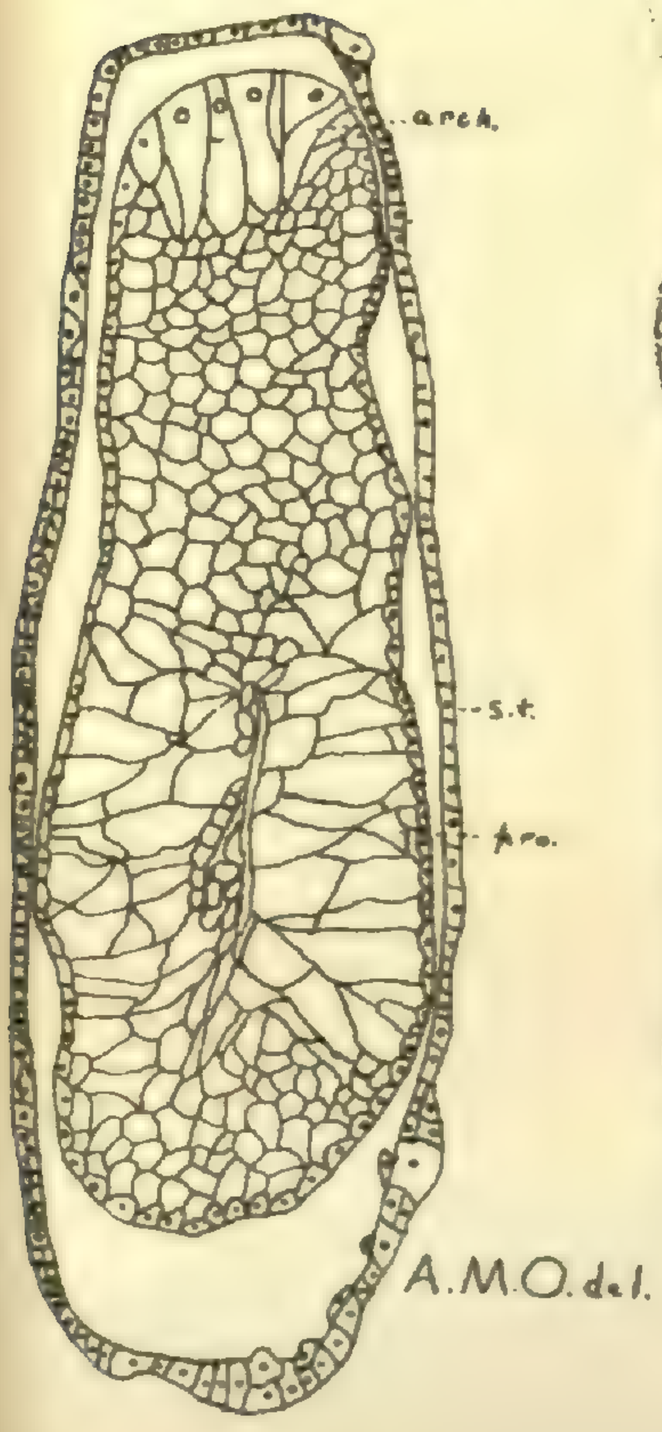

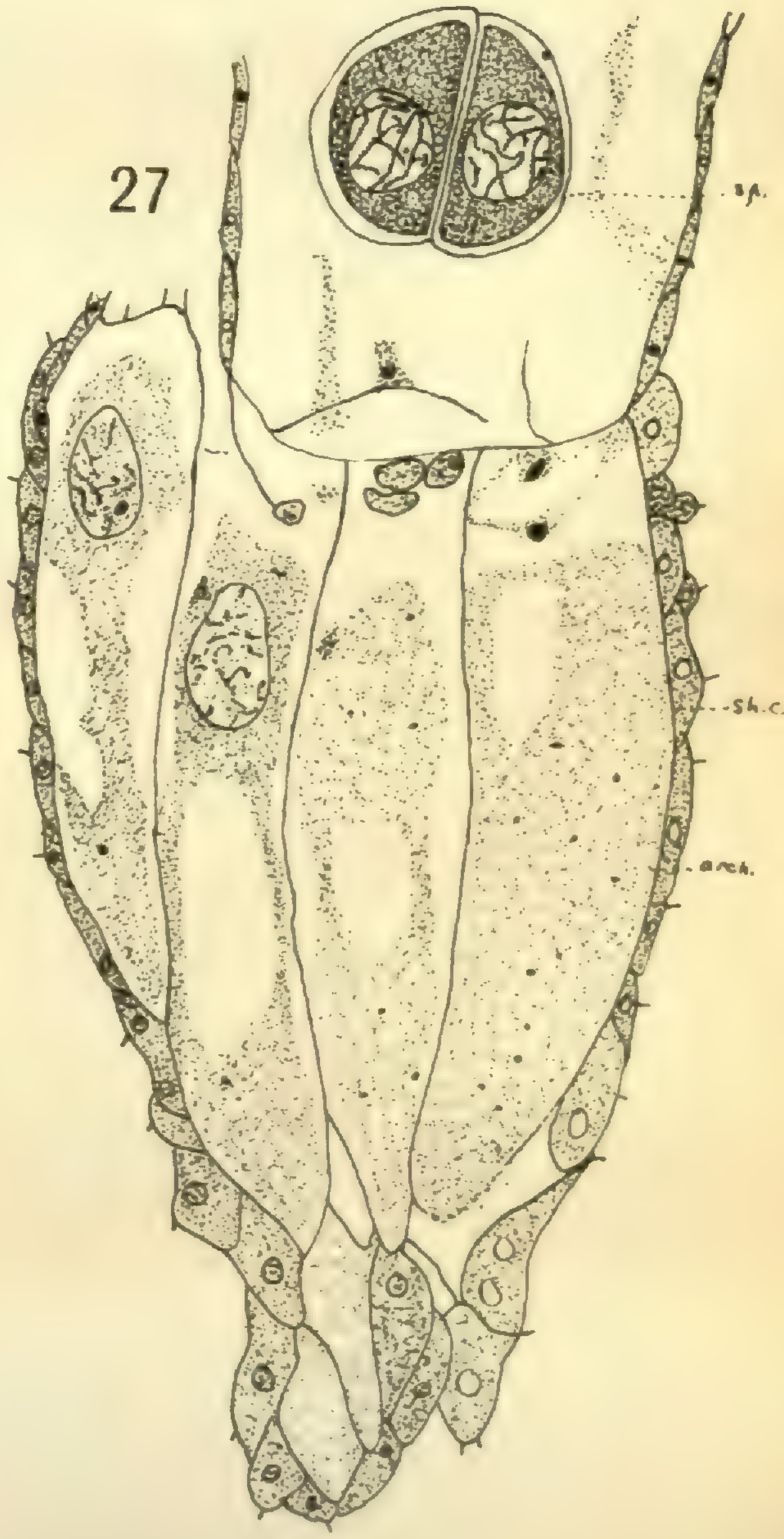

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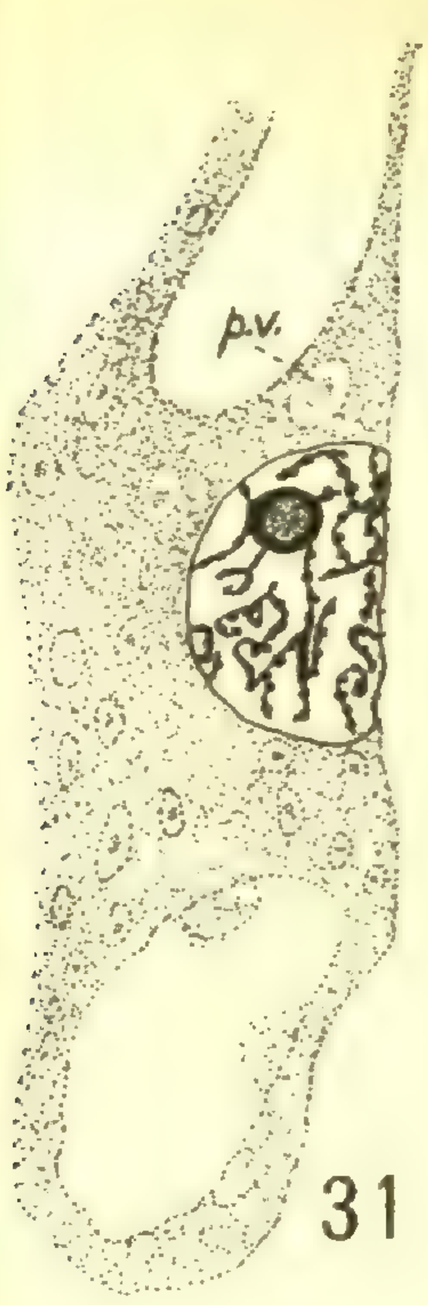

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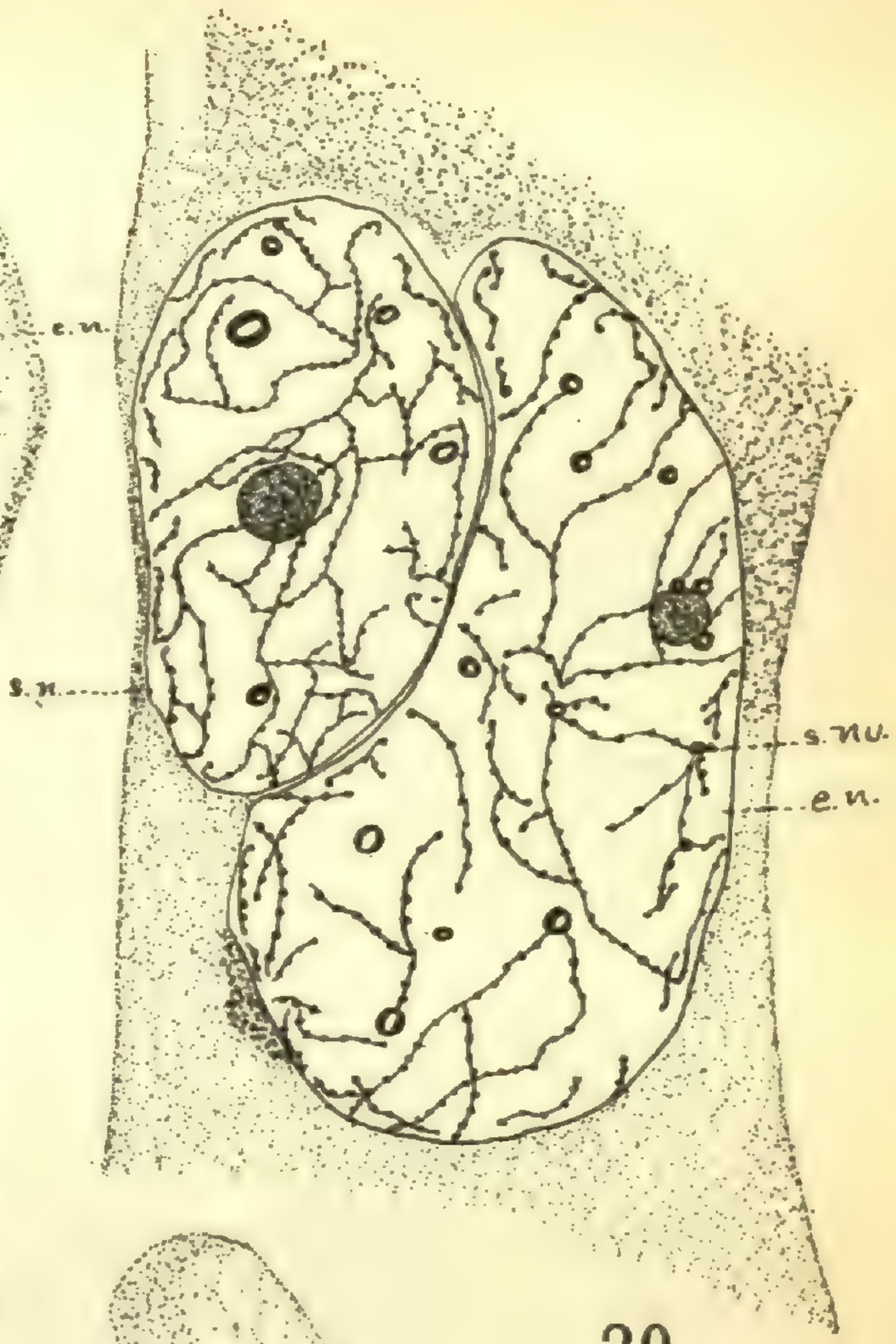

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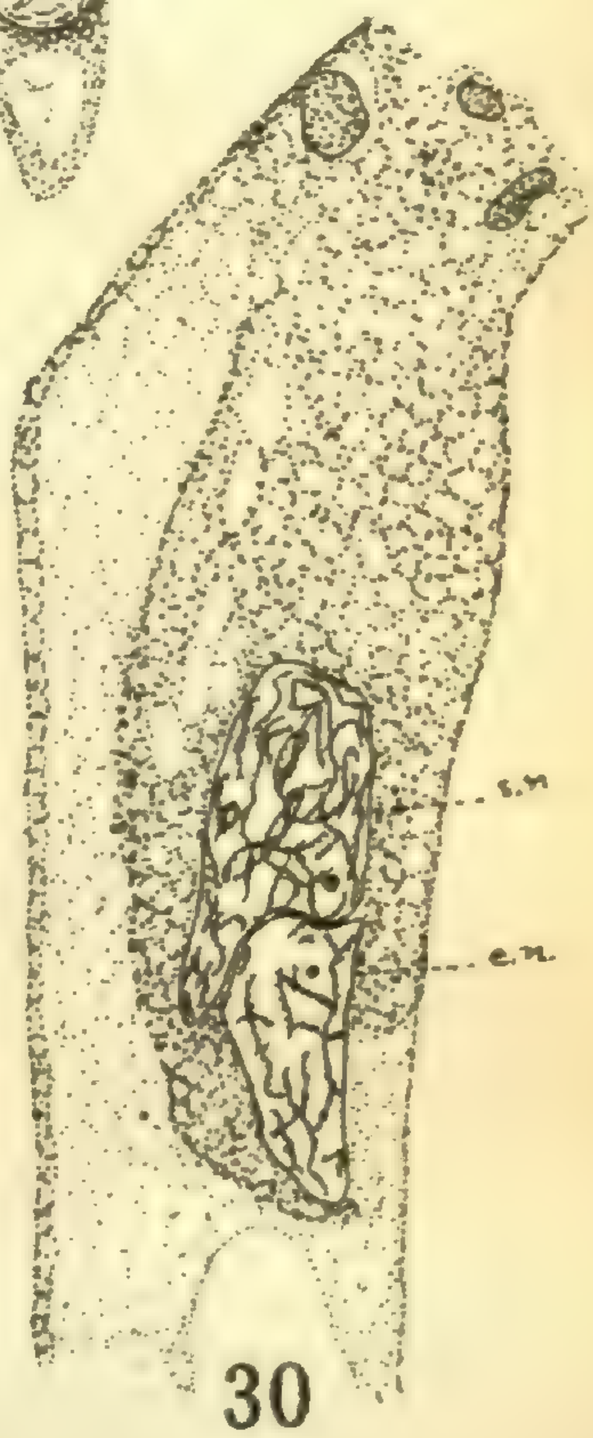

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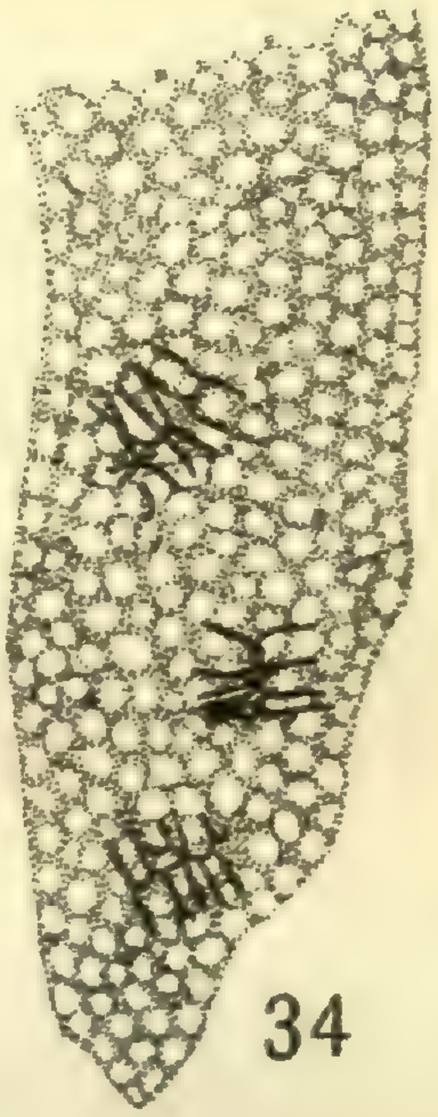


34

OTTLEY on JUNIPERUS

THE STRUCTURE OF THE WOOD IN THE PINEAE[1]

IRVING W. BAILEY

(WITH PLATE V)

The Abietineae have been conveniently divided into the subfamilies Pineae and Abieteae.[2] The former are characterized by the presence of resin canals in the xylem, by their thinly integumented ovules, and by the non-deciduous character of the cone scales. The Abieteae differ in having resin canals absent from the woody tissues of the stem (except in the first annual ring of vigorous shoots of vigorous specimens of Abies), and by possessing usually a thick-coated ovule and deciduous cone scales. In both subfamilies resin canals may occur traumatically. These are easily differentiated from those normal to the stem by the fact that they occur in tangential rows of numerous canals, intercommunicating tangentially, and composed of generally heavily pitted epithelial cells. The Pineae comprise the genera Pinus, Picea, Larix, and Pseudotsuga. The Abieteae include Abies, Tsuga, Cedrus, and Pseudolarix. It will be the object of the present article to consider the structure of the woody cylinder in the former subfamily.

The four genera of the Pineae have been classified, by various authorities, according to the anatomical structure of the xylem as follows.

PINUS

The wood is characterized by the presence of numerous resin canals with thin-walled epithelium. This condition is constant except in the nut pines and foxtail pines of the southwestern United States, where thick-walled epithelial cells are interspersed among the usually thin-walled type. Further, Pinus is supposed to be characterized by the entire absence of wood-parenchyma, and according to GOTHAN of spiral thickenings of the tracheid walls of the secondary wood.[3]

[1] Contributions from the Phanerogamic Laboratories of Harvard University, No. 15.

[2] JEFFREY, The comparative anatomy and phylogeny of the Coniferales. Part 2. The Abietineae. Mem. Boston Soc. Nat. Hist. **6**:21. 1905.

[3] GOTHAN, Zur Anatomie lebender und fossiler Gymnospermen-Hölzer. Abhandl. König. Preuss. Geol. Landesanstalt. Neue Folge, Heft 44, p. 99. Berlin. 1905.

The pines are divided into the hard pines and the soft pines. The former are characterized by possessing dentate or reticulate tracheids of the rays, and by having no tangential pits developed in the tracheids at the end of the year's growth. The soft pines, on the contrary, are supposed to possess marginal and interspersed tracheids of the rays with unsculptured walls, and to have well-developed tangential pits in the tracheids formed at the end of the year's growth. The majority of the hard pines and soft pines can easily be separated from the remaining genera of the Pineae by the character of the pits in the lateral walls of the parenchymatous cells of the rays. These pits are very large and often polygonal, and quite distinct from the small round pits of Picea, Larix, and Pseudotsuga. However, in the nut and foxtail pines above referred to, and in certain of the hard pines, we find a diminution in the size of the pits, which approaches the condition in the Picea type.

PICEA

Picea has a wood with thick-walled epithelial cells in the resin canals and tangential pits in the summer tracheids. The pits in the lateral walls of the ray cells are small and round. The wood-parenchyma is entirely absent. Spiral thickenings of the tracheids are also absent, according to PENHALLOW.[4] However, GOTHAN (*op. cit.* 98) and other European authorities note their presence in the summer wood alone.

LARIX

The wood resembles Picea as regards the thick-walled character of the resin canals, the presence of tangential pits in the summer tracheids, and the small size of the lateral pits of the ray cells; but possesses well-developed wood-parenchyma upon the outer surface of the summer wood. Spiral thickenings of the tracheids are present only in the summer wood.

PSEUDOTSUGA

The wood resembles Picea and Larix as regards thick-walled epithelium, tangential pits of the summer tracheids, and form and size of the pits of the ray cells. It also resembles Larix in having parenchyma well developed upon the outer surface of the summer wood. The wood, however, is supposed to be quite distinct in having well-

[4] PENHALLOW, North American Gymnosperms 195. Boston. 1907.

developed spiral thickenings in the spring as well as the summer tracheids. Further, according to MAYR,[5] spirals are also present in the ray tracheids of *Pseudotsuga macrocarpa* Mayr, but not in *P. Douglasii* Carr.

With this review of the generally accepted classification of the Pineae, let us turn to a careful consideration of the occurrence of each of the anatomical characters referred to above. In the first place as regards

WOOD-PARENCHYMA

As stated above, these cells are supposed to exist on the outer surface of the summer wood in Larix and Pseudotsuga, and to be entirely absent from Pinus and Picea. The presence of wood-parenchyma on the face of the summer wood in the xylem of a specimen of *Picea excelsa* Link in the Harvard Botanical Gardens led me to study this tree, believing that it was an unusual or freak specimen. The examination of sections from the root and stem showed the parenchyma well developed at the end of the year's growth (*figs. 1–3*), but in many cases the parenchymatous cells were rare or apparently absent from the stem. However, numerous sections cut from the same piece of wood always revealed one to several cells. It is difficult to see them in a transverse section, as they can be distinguished with certainty only when the simply pitted walls are visible. Obviously, to obtain this condition when the cells are rare is difficult. Likewise, in radial sections it is not easy to find them. If, however, tangential sections are cut in a slightly oblique manner, several layers of summer wood are visible in a single section. Many sections cut in series would then be more liable to reveal the presence of parenchymatous cells even when occurring infrequently. Cutting the sections in series avoids the possibility of confusing the parenchyma, for which I was searching, with that associated with resin canals or wound callus. However, the appearance of the latter as well as their location (not confined to the outer layer of the summer wood) is sufficient for their identification without these precautions.

I decided to use this method in the examination of other specimens of *Picea excelsa*, to see if wood-parenchyma were characteristic of the species. Upon examination it was found that the cells were present

[5] MAYR, Die Waldungen von Nordamerikas 424. *pl. 9.* München. 1890.

in *Picea excelsa* vars. *monstrosa*, *conica*, *elata*, *pendula*, and *pyramidalis*. Finding them characteristic of these varieties of the commonly planted *Picea excelsa*, it was decided to extend the studies to other species. Through the courtesy of Professor JACK, of the Arnold Arboretum, I was able to secure carefully identified green material of seventeen species of American, Asiatic, and European spruces. Thin serial sections of this material showed that the presence of wood-parenchyma upon the outer surface of the summer wood is a characteristic condition for Picea. Its occurrence, however, is extremely sporadic. In any given fragment of wood the cells may or may not appear, and while usually occurring very infrequently, may at any time appear in large numbers. These cells appeared more numerously in the European and Asiatic species, and in *Picea sitchensis* Carr. and *Picea Parryana* Sarg. of the American species. In the spruces from the northeastern United States they could be made out only with difficulty.

The extremely variable occurrence of wood-parenchyma is also characteristic of Larix and Pseudotsuga, for though usually occurring numerously upon the outer surface of the summer wood, in many specimens they may be very infrequent or nearly absent.

SEPTATE TRACHEIDS

In the three genera of the Pineae just mentioned septate tracheids occur with the parenchyma upon the outer surface of the year's growth. In Picea they are usually more numerous than the wood-parenchyma and occur where wood-parenchyma is not developed. In Larix and Pseudotsuga the parenchyma usually predominates. There is a clear gradation shown from tracheid to parenchyma in these genera. Frequently one may observe in the same section a septate tracheid, a tracheid partly septate and partly parenchymatous (*fig. 4*), and a series of resin cells, together having the form of a tracheid. In other words, the various steps by which tracheids have been modified to form parenchyma are clearly shown. In support of these statements it may be well to add that primitive woods were composed entirely of tracheids and medullary parenchyma, and that only in the higher gymnosperms do we see the development of wood-parenchyma, first upon the outer surface of the summer wood, and later throughout

the year's growth in plants which have lost, or are in the process of losing, their resin canals. In the Pineae, in which resin canals occur normally, the parenchyma is confined to the end of the year's growth, and less well developed in the older genera, which have more numerously developed resin canals. Thus Pinus, with its large supply of resin canals, shows only the first steps in the development of wood-parenchyma. Septate tracheids occur infrequently upon the outer surface of the summer wood, and only in one instance have I been able to observe what appeared to be parenchyma in the same location. In Picea, with less well-developed resin ducts, the wood-parenchyma becomes constant, while in Larix and Pseudotsuga it is usually strongly developed. In the Taxodineae and Cupressineae, with the nearly complete disappearance of resin canals, the wood-parenchyma is well developed throughout the year's growth, as well as at the end of the summer wood. The Abieteae are transitional between the two groups. It seems probable, as JEFFREY has suggested in his paper on the Abietineae (*op. cit.* 26), that with the reduction of foliage in the Abietineae and Cupressineae, the freely anastomosing system of canals, with its large supply of resinous secretions for sterilizing wounds, became too great a burden, and that the system of resin cells was gradually developed to take its place. It is not my object to enter here into all the various phases of the controversy as to the age of the Abietineae, but recent paleontological evidence proves the great geological age of Pinus. Further, Prepinus is a primitive abietineous type with centripetal wood, resembling certain of the Cordaites, and at the same time closely allied to the true pines of the middle Cretaceous.[6] A survey of all the paleontological evidence, as well as a study of the anatomy and morphology of the modern Coniferales, seems to show conclusively the greater age of the Abietineae.

SPIRAL THICKENINGS

Let us now turn to a consideration of the occurrence and distribution of tertiary thickenings of the tracheid walls in the Pineae.[7] As stated above, PENHALLOW considers spiral thickenings absent from

[6] JEFFREY, Structure of the leaf in Cretaceous pines. Annals of Botany 21:207–220. *pls. 13, 14.* 1908.

[7] It should be kept clearly in mind that these spirals are tertiary thickenings, and are entirely distinct from the so-called "spiral striations" of the secondary walls of the tracheids.

Picea, while European authorities note their presence only in the summer wood. In the seventeen species of Picea which I examined, I found spirals well developed in the summer wood of the first few years' growth, usually from the first or second to the tenth year. In older woods they are very sporadic in their development, in certain regions appearing well developed, but usually showing at best only feebly. In wounded areas they showed a tendency to be strongly developed. In two species, *Picea sitchensis* Carr. and *Picea Maximowiczii* Regel, the spirals were very strongly developed in the spring as well as the summer wood (*fig. 6*). Furthermore, in both of these species, spiral thickenings were well developed in the marginal and interspersed tracheids of the rays (*fig. 5*). They occurred in the ray tracheids in both the summer and spring wood. In other species of Picea this condition could be made out in the tracheids of the rays of the summer wood. In other words, the ray tracheids appear to follow closely the wood tracheids. Where spirals are strongly developed in the latter elements, they will appear usually in the former.

In Larix the spirals were observed occurring in the first few years' growth, but not so well developed as in Picea. In the mature wood and in the ray tracheids, the spirals are likewise sporadic in their appearance, and may or may not be noticeably developed. Only four species were examined, *Larix americana* Michx., *L. europaea* DC., *L. occidentalis* Nutt., *L. dahurica* Turcz., and in none of these were spirals present in the spring wood; yet from the similarity to Picea and Pseudotsuga I should consider it extremely likely that they would be found, if a large amount of material were examined.

In Pseudotsuga spirals occur in both the summer and spring tracheids, yet they also are sporadic in their development, in certain specimens appearing well marked throughout, or in others nearly absent. MAYR notes the presence of spiral thickenings in the marginal tracheids of *Pseudotsuga macrocarpa* Mayr, but states (*loc. cit.*) that they are absent in *P. Douglasii* Carr. Sections from specimens of the Douglas fir from various sources show the spirals well developed in the marginal tracheids of certain specimens and less well developed in others.

In Pinus, as has been noted, GOTHAN asserts the absence of spirals, yet PENHALLOW (*op. cit.* 41) has described them as occurring in

Pinus taeda Linn., and I have myself observed them in *Pinus attenuata* Lemm. and several other species. Furthermore, spiral thickenings occur in the ray tracheids of *Pinus Balfouriana* A. Murr.; they are strongly developed in certain specimens and only feebly in others. *Pinus strobiformis* Engelm. and other pines from the southwestern United States show traces of their occurrence.

From the development of the spirals of the tracheids in the first year's growth and in areas of injury, it would appear that the spirals were an early development, yet the fact that they are not always present in the first and second annual rings, and are absent from the axis of the cone, would seem to show that the character cannot be primitive. It certainly would not be safe to assume that the presence of these spirals is an indication of close relationship with the Taxaceae, for spiral thickenings of the vessels in the dicotyledons are found in such widely separated families as the Betulaceae and Tiliaceae.

INSTABILITY OF DIAGNOSTIC CHARACTERS

From the preceding remarks on the development of wood-parenchyma and spiral thickenings, and the sporadic and uncertain character of their appearance, one realizes the difficulty in finding any definite basis upon which to separate Picea, Larix, and Pseudotsuga. As has been shown, one can no longer depend upon the presence or absence of wood-parenchyma and spirals to acomplish this end.

Let us now turn to consider the stability of the other elements of the wood. The size, form, and location of the resin canals vary so greatly in material from different sources that no rule can be formulated to cover every case. It is true that Picea approaches nearer to the condition found in the nut and foxtail pines than the other genera. Thus certain spruces have considerable thin-walled epithelium and tyloses, yet thin-walled epithelium occurs in Larix and Pseudotsuga. Further, the same variability is characteristic of the pitting of the rays and tracheids, and of the weight and color of the wood. We are thus forced to the conclusion that in order to distinguish the woods of Picea, Larix, and Pseudotsuga, a careful study must be made of all the anatomical and gross characters, and comparisons made with type sections. In other words, one must apply here the same methods in differentiating genera which one usually uses in distinguishing species.

As an illustration of the difficulty in distinguishing these woods let us consider a fossil from California recently described by GOTHAN.[8] He identifies the specimen as follows. The fact that the resin canals are typically non-pine-like, with thick-walled epithelium, shuts out Pinoxylon. The presence of wood-parenchyma and spirals in the spring wood shows its relation to Pseudotsuga. *Pseudotsuga macrocarpa* has ray tracheids with spirals, and in *P. Douglasii* they are absent. Thus the fossil, which he calls *Piceoxylon Pseudotsugae*, as it has no spirals in the ray tracheids, must be closely allied to the Douglas fir, if not a fossil specimen of it.

In the first place, as we have seen, *Pseudotsuga Douglasii* possesses spiral thickenings in the ray tracheids. This, according to the author's own line of reasoning, would exclude *P. Douglasii.* Further, let us consider the statements in regard to the presence of wood-parenchyma and spiral thickenings in the spring wood. As has been shown above, both these conditions occur in *Picea sitchensis*, a spruce from the Pacific coast. Can we be sure whether this fossil is more closely allied to Pseudotsuga, Picea, or even Larix? As an added reason for regarding the fossil allied to Pseudotsuga, the author states that the horizontal resin canals in the fusiform rays occur in an unsymmetrical manner. This same condition may be frequently observed in both Picea and Larix. One comes to the conclusion that the identification of woods and fossils of Picea, Larix, and Pseudotsuga is an extremely difficult undertaking.

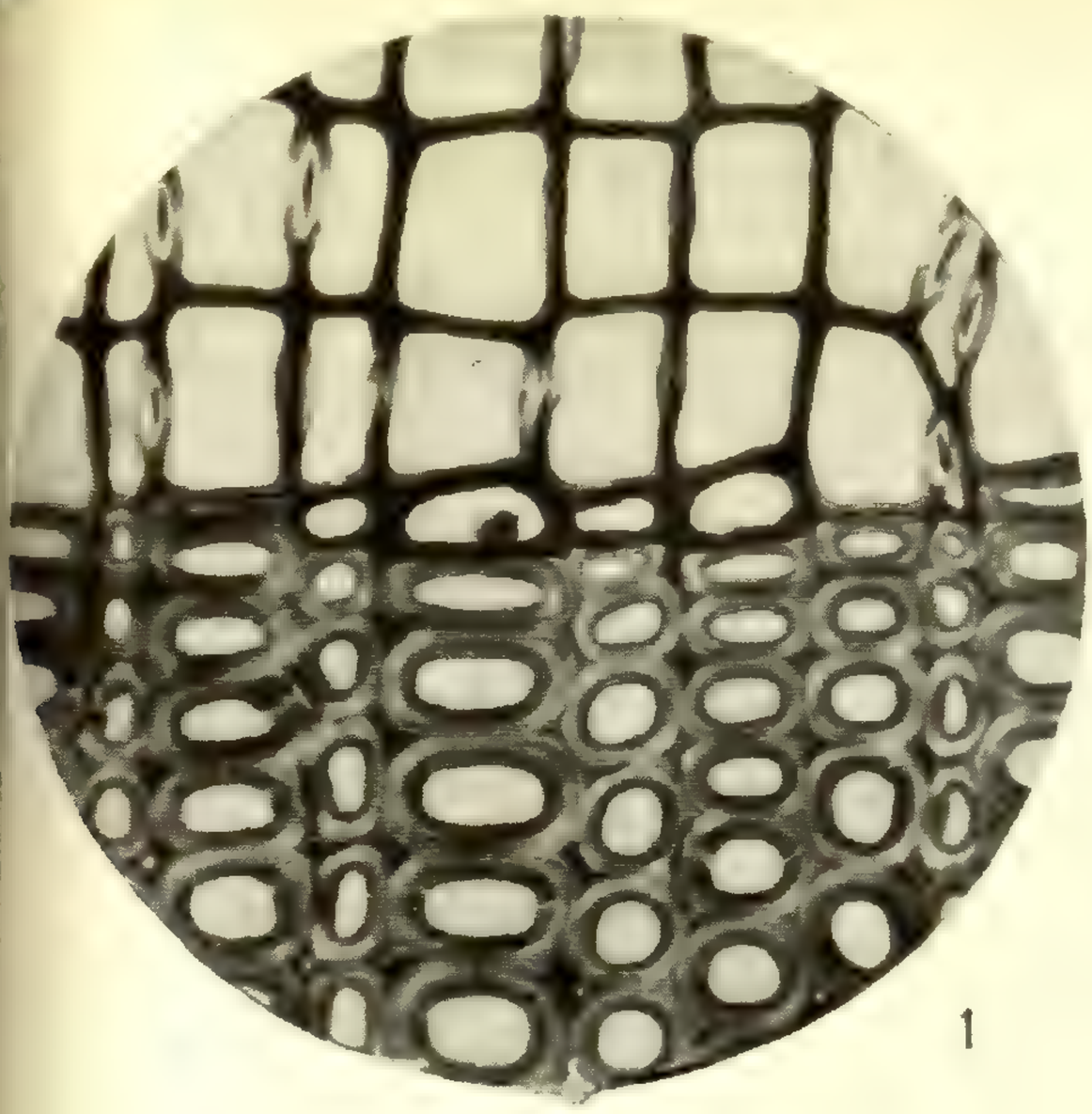
SUMMARY

1. Wood-parenchyma occurs on the outer surface of the summer wood of Picea. It is sporadic in its occurrence, and while usually appearing infrequently, may be strongly developed.

2. Wood-parenchyma may be very sparsely developed in Larix and Pseudotsuga.

3. Septate tracheids occur associated with the wood-parenchyma in these three genera, and show clearly the steps by which wood-parenchyma has been developed from tracheids.

[8] GOTHAN, *Piceoxylon Pseudotsugae* als fossiler Holz, *Pseudotsuga* sp. (aff. *Douglasii*) als rezenter Baum; in H. POTONIE, Abbildungen u. Beschreibungen Foss. Pflanzen, Lief 4. 1906.



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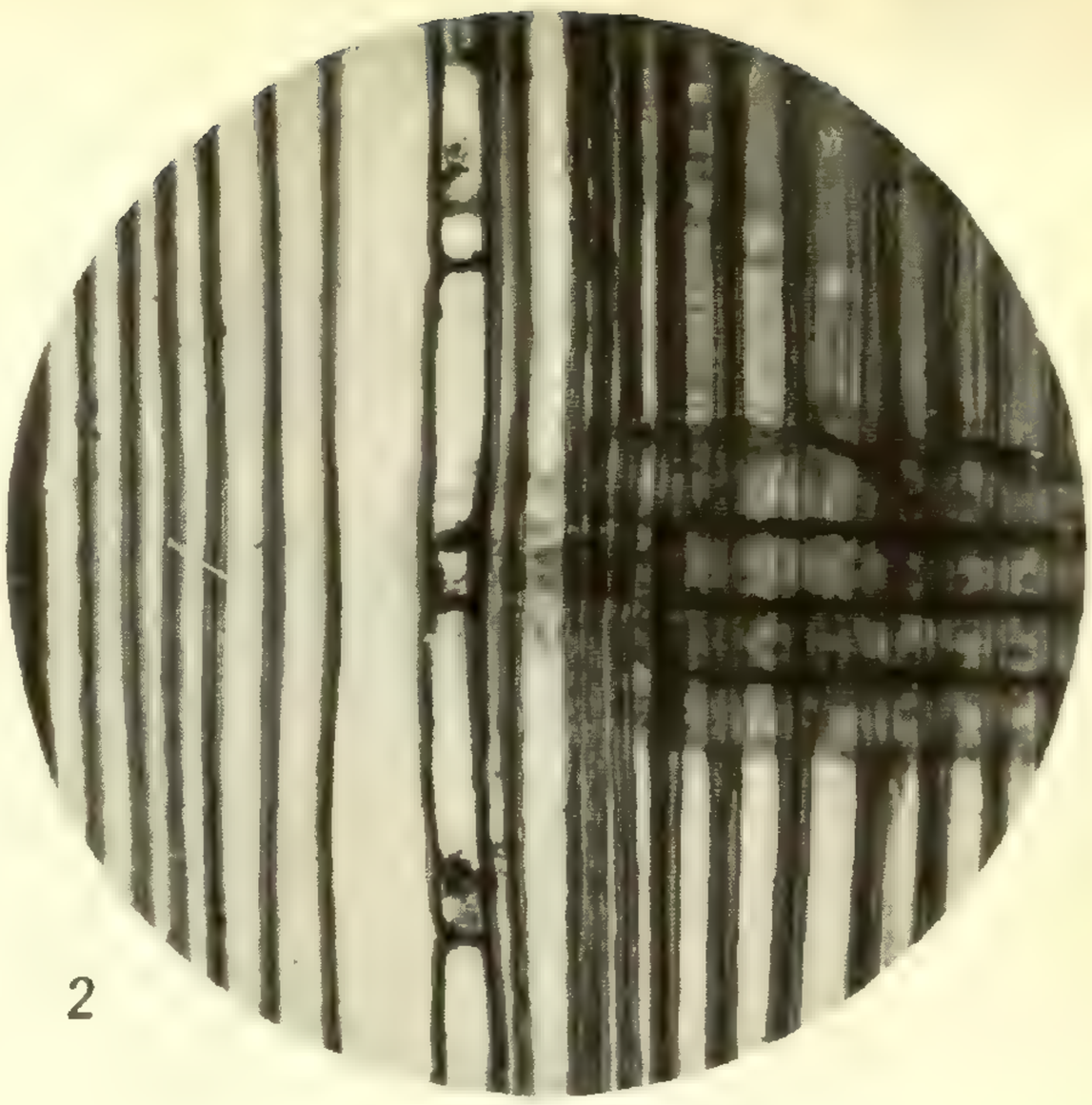

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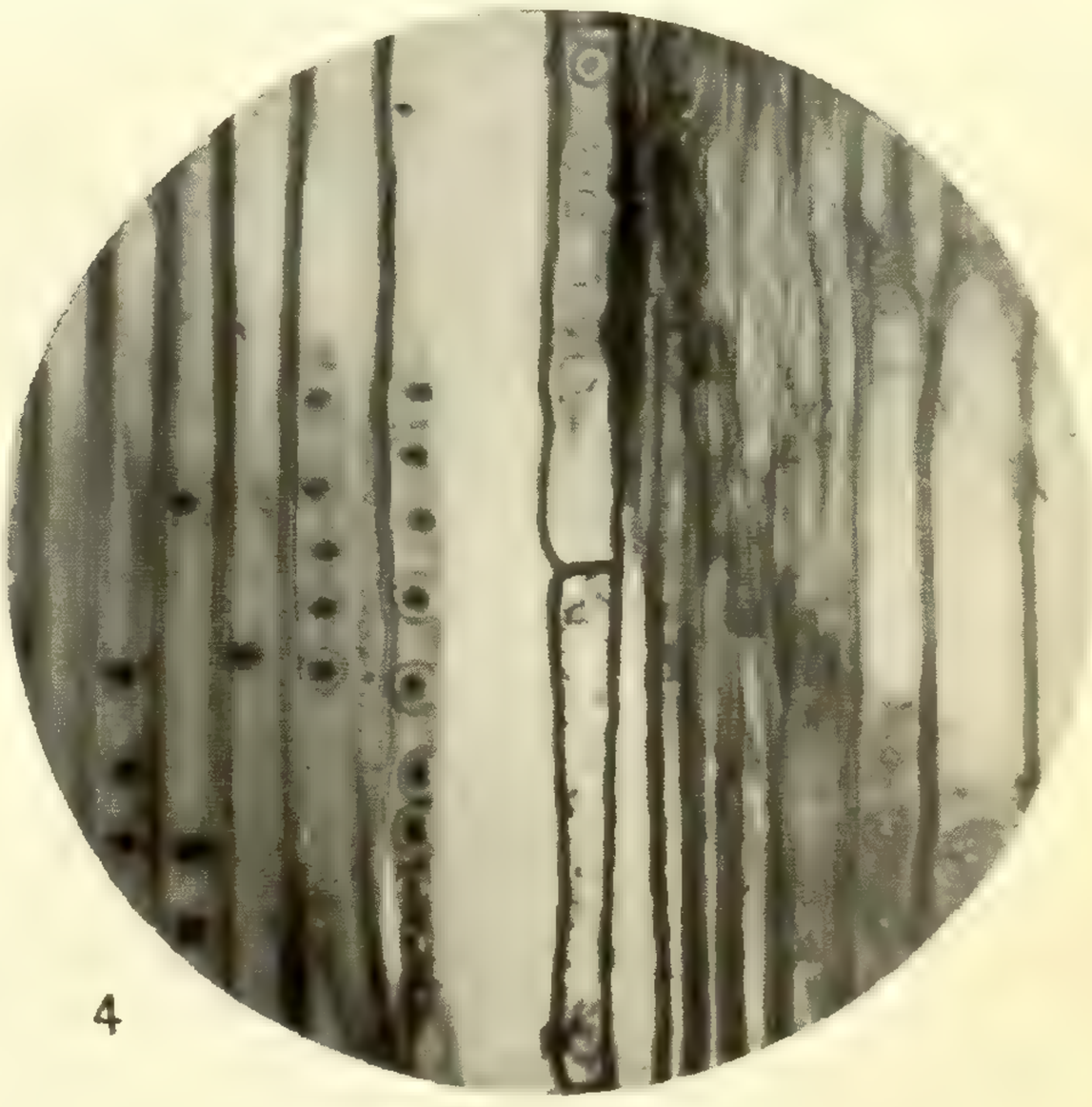

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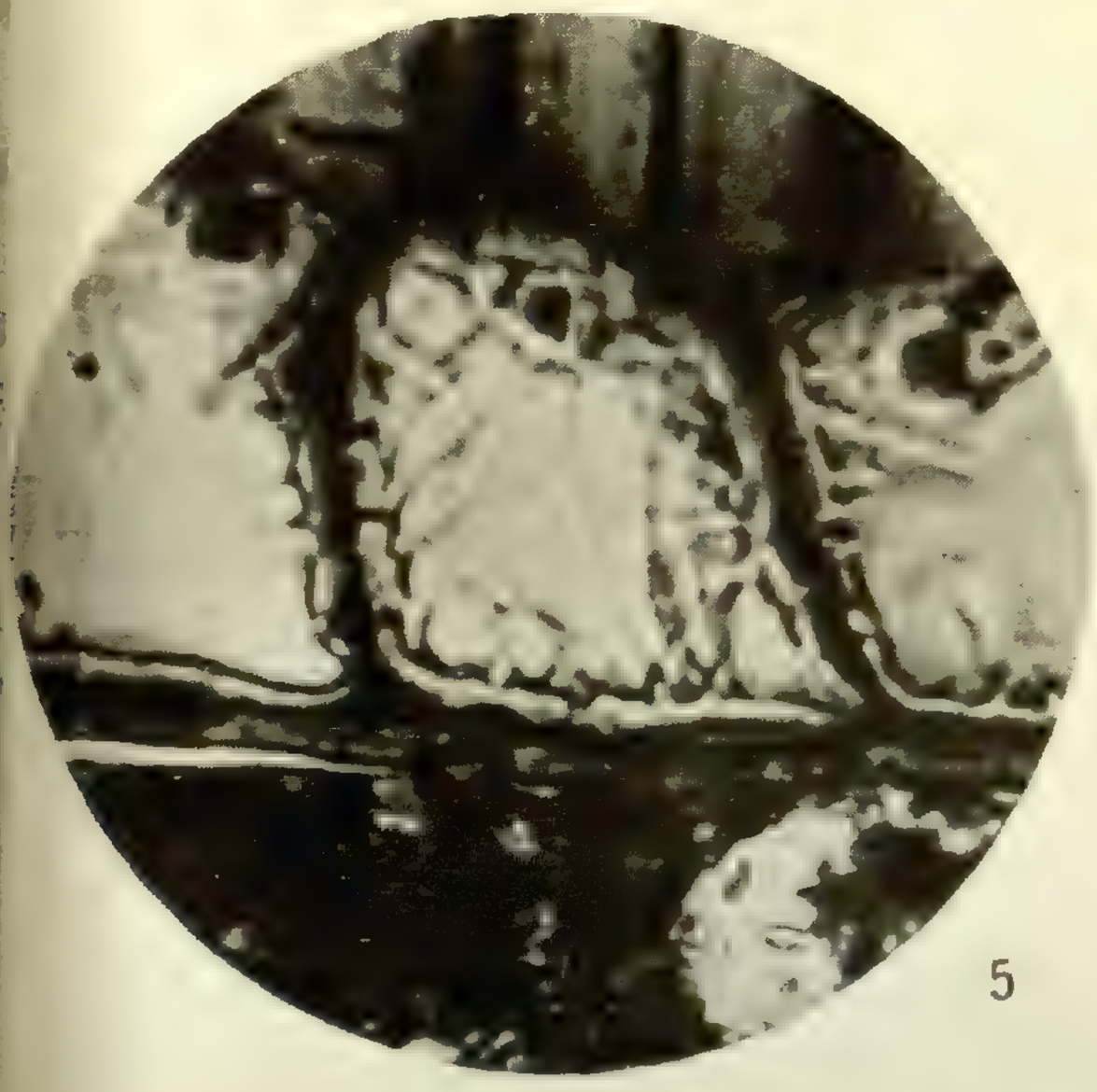

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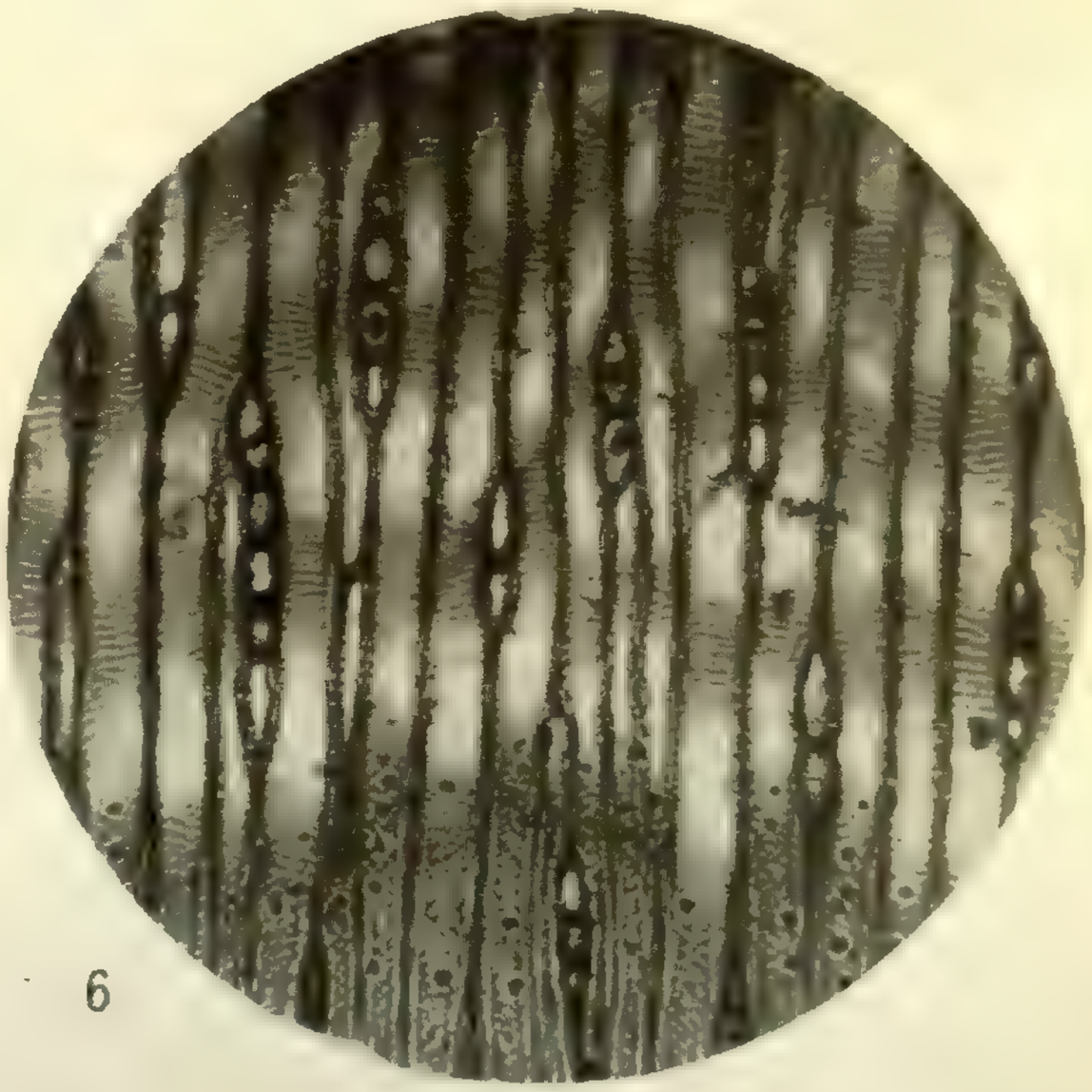

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BAILEY on WOOD OF PINEAE

4. Septate tracheids occur in Pinus, and wood-parenchyma very rarely.

5. Spiral thickenings of the tracheids occur in both the spring and summer wood in Picea and Pseudotsuga, and occur in the summer wood of Larix. These thickenings occur also in Pinus.

6. Spiral thickenings of the marginal and interspersed tracheids of the rays occur in Pinus, Picea, and Larix, as well as in Pseudotsuga.

7. The anatomical characters of the wood are so variable and so similar in Picea, Larix, and Pseudotsuga, that it is difficult to distinguish the extant or fossil woods of the genera.

8. Pinus appears to be quite distinct from the other living Pineae. Yet in the nut and foxtail pines, we see a condition resembling the condition in Picea, Larix, and Pseudotsuga. These pines have small rounded pits in the ray cells, have tangential pits like Picea, have thick-walled epithelium in the canals, and spiral thickenings in the ray tracheids.

9. Picea approaches nearer to the condition in Pinus by having more numerous thin-walled epithelial cells and tyloses, and less well-developed wood-parenchyma; yet Larix and Pseudotsuga also have thin-walled epithelial cells occasionally, and may have the wood-parenchyma poorly developed.

HARVARD UNIVERSITY
CAMBRIDGE, MASS.

EXPLANATION OF PLATE V

FIG. 1.—Transverse section of stem of *Picea excelsa*, showing four resin cells on the outer face of the summer wood. ×500.

FIG. 2.—Radial section of root of *Picea excelsa*, showing resin cells on the outer face of the summer wood. ×180.

FIG. 3.—Tangential section of root of *Picea excelsa*, showing resin cells. ×180.

FIG. 4.—Radial section of root of *Picea excelsa*, showing tracheid partly septate and border-pitted, and partly parenchymatous and simply pitted. ×180.

FIG. 5.—Radial section of stem of *Picea Maximowiczii*, showing spiral thickenings in the marginal tracheids. ×900.

FIG. 6.—Tangential section of stem of *Picea Maximowiczii*, showing spiral thickenings in both the spring and summer wood. ×180.

BRIEFER ARTICLES

NOTES ON THE EMBRYOLOGY OF THE NYMPHAEACEAE

(WITH PLATE VI)

Within the past few years the embryology of the Nymphaeaceae has attracted considerable attention, beginning with LYON'S studies on Nelumbo,[1] followed by my own paper on *Castalia odorata* and *Nymphaea advena*[2] and by CONARD'S[3] monograph on the water lilies. The differences of opinion as to certain characters of the embryo, expressed by CONARD on the one side and LYON and myself on the other, led me to believe that the embryological characters are subject to considerable variation, or that some serious error had been made. However, I determined to verify my work on *Castalia odorata* and *Nymphaea advena*, if possible, and YORK'S[4] work on Nelumbo. Recently SEATON[5] has published a paper on *Nymphaea advena*, which also deserves consideration in this review of the subject.

My recent studies have been confined to the disputed points in *Castalia odorata*, *Nymphaea advena*, and *Nelumbo lutea*, and therefore other species and other questions have been given very little or no consideration.

EMBRYO SAC

The mature embryo sac is subject to considerable variation. However, I am now convinced that the formation of a cross-wall between the two daughter nuclei, which are formed by the first division of the endosperm nucleus, is a constant character, although SEATON failed to find it in *Nymphaea advena*. It is usually very delicate and is no doubt frequently

[1] LYON, H. L., Preliminary note on the embryogeny of Nelumbo. Science N. S. 13:470. 1900.

———, Observations on the embryogeny of Nelumbo. Minn. Bot. Stud. 2:643–655. 1901.

[2] COOK, M. T., Development of the embryo sac and embryos of *Castalia odorata* and *Nymphaea advena*. Bull. Torr. Bot. Club 29:211–220. 1902.

[3] CONARD, H. S., The water lilies: a monograph of the genus Nymphaea. Carnegie Inst. Wash. Publ. 4. 1905.

COOK, M. T., The embryology of some Cuban Nymphaeaceae. BOT. GAZETTE 42:376–392. 1906.

[4] YORK, H. H., The embryo sac and embryo of Nelumbo. Ohio Nat. 4:167–176. 1904.

[5] SEATON, SARA, The development of the embryo-sac of *Nymphaea advena*. Bull. Torr. Bot. Club 35:283–290. 1908.

destroyed in the preparation of the material. The writer has observed it repeatedly in *Castalia odorata* and in *Nymphaea advena*, where it can be seen without great difficulty; also in *Cabomba piauhiensis* and in *Brasenia purpurea*, where it is very difficult to demonstrate. YORK noted it in *Nelumbo lutea*, but according to his observations one of these two cells again divides, thus forming three superposed cells, each of which is active in the formation of the endosperm. However, he did not observe the formation of the large vesicular cell, which is so characteristic of the other genera of this family. My recent studies, which will be referred to later in this paper, do not fully agree with those of YORK in regard to the vesicular cell.

The daughter nucleus in the micropylar end of the sac always divides to form the endosperm. The formation of the nucellar tube, into which the other nucleus usually and possibly always passes, is subject to considerable variation in the different species. In the genus Nymphaea it is a cylindrical tube, which extends almost the entire length of the ovule (*fig. 1*). In Castalia it may be in the form of a tube which is practically the same as in Nymphaea, but it is usually somewhat shorter and narrower. In some of my recent studies I have found individuals in which there were well-formed tubes extending only about one-half the length of the ovule (*fig. 2*), while in other individuals it extended not more than one-third the length of the ovule and tapered to a point (*fig. 3*). In *Castalia ampla*, as previously reported by me, it develops into a short, sac-like structure (*fig. 4*), which is eventually overgrown by the micropylar part of the sac (*fig. 5*). In *Brasenia purpurea* and *Cabomba piauhiensis*, the tube is very long, extending almost the entire length of the ovule, and very small (*fig. 6*). In *Nelumbo lutea*, according to these studies, the tube is very irregular in form and extends about two-thirds the length of the ovule (*fig. 7*).

In these recent studies one individual of *Nelumbo lutea* was observed in which two sacs were formed one below the other in the main axis of the ovule (*fig. 8*). The antipodal ends were in contact, and the sac next to the micropyle was about four times as large as the other. It showed a fully developed egg-apparatus, and the two polar nuclei were in contact, but the antipodals had disintegrated. The small sac which was farthest from the micropyle showed the egg, one synergid, the three antipodals, and was filled with the characteristic endosperm. In the antipodal end of the sac was a small, deeply colored mass, which was possibly the remains of the disintegrating tube nucleus (*fig. 8 t*). This extra sac was evidently derived from one of the sister megaspores. YORK observed many cases in which extra, imperfect sacs were produced, and expressed the opinion that they were from sister megaspores rather than from independent mega-

sporocytes. I have previously recorded the occurrence of extra, imperfect sacs in Castalia, Brasenia, and Cabomba, but in all cases I considered them to have been derived from independent archesporial cells. In this new case, to which I now refer, it was evident that the second sac had not been penetrated by a pollen tube, and of course the endosperm had been formed without triple fusion. In this connection I wish to say that I have previously observed sacs of species of this family which were filled with typical endosperm, but without embryos, and in which it appeared that there had been no fertilization.

However, in the normal embryo sacs of *Nelumbo lutea*, the polar nuclei united in the usual manner and then divided to form the endosperm. I was unable to follow the early divisions of the endosperm, but in a few instances I found a well-marked cell wall across the sac, which I take to be the wall that is usually formed between the two daughter nuclei after the first division of the endosperm (*fig. 10*). The tube nucleus (*t*) was comparatively small and undergoing disintegration. The antipodal end of the sac is in contact with an axial mass of cells (*fig. 11*), which may consist of a single row of cells or many rows. These cells are rich in protoplasm and eventually disintegrate, probably forming food for the developing embryo. They have been referred to by YORK, who gave a discussion of their appearance and behavior, and figured them in his paper. They are very similar to the cells in the secondary sac (*fig. 8*), and I am inclined to consider them as the vestiges of undeveloped embryo sacs. Near the cross-wall, but in the micropylar chamber of the sac shown in *fig. 10*, was a rather compact mass of cells (*d*), which somewhat resembles a second embryo, but a careful examination leads me to believe it a part of the endosperm.

In one ovule of *Castalia odorata* the embryo was well advanced, although the endosperm nucleus had remained undivided (*fig. 13*).

EMBRYOS

In my first paper I stated that the embryos are without suspensor, except in the case of *Nymphaea advena*, in which a latent suspensor is developed. CONARD afterward described and figured a suspensor for Castalia. In a more recent paper on the Cuban forms, I described the embryo of *Nymphaea advena* as developing in the same manner as our northern form, except that the latent suspensor was more prominent in the Cuban form. However, the embryos of *Castalia ampla* and *C. pubescens* develop with filamentous suspensors in the manner described by CONARD for *Castalia odorata*, while Cabomba and Brasenia both develop with short fila-

mentous suspensors. SEATON's observations on the embryo of *Nymphaea advena* coincided with my first paper on this subject.

In these later studies I have found that the embryo of *Nymphaea advena* originated (*fig. 12*) and develops in the same manner as previously described by me, but that in some instances the latent suspensor is almost or quite as prominent as in the Cuban form. In *Castalia odorata* I found both types of embryos described by me in my first paper; i. e., without a suspensor (*fig. 13*), and also with a suspensor (*fig. 14*), as described by CONARD and by myself in my paper on the Cuban Nymphaeaceae. These recent studies on *Nelumbo lutea* showed in most cases a spherical embryo, without a suspensor, and agreed fully with the observations of LYON and YORK, but in one or two cases there appeared a small suspensor (*fig. 10*) of the same type as described for *Nymphaea advena*, except that it appeared somewhat earlier in the development of the embryo.

Recent studies on *Castalia odorata* and *Nymphaea advena* confirm the previous observations of myself and others on the formation of a cotyledonary ridge, from which the two cotyledonary lobes are developed.

The studies referred to in this paper convince me that the species of Nymphaeaceae are either very plastic and subject to considerable variation, or that we are confusing very closely related forms.

SUMMARY

1. Extra embryo sacs are frequently formed.

2. The cross-wall between the two daughter nuclei, which are formed by the first division of the endosperm nuleus, is usually present, although so delicate that it is very difficult to demonstrate.

3. The nucleus in the micropylar end of the sac forms the endosperm.

4. The nucellar tube is somewhat different in the different species, and also subject to great variation, especially within the genus Castalia.

5. The embryo of Nymphaea originates without a suspensor and later develops a latent suspensor; the embryo of Castalia originates either without or with a filamentous suspensor; the embryos of Cabomba and Brasenia with short filamentous suspensors; and the embryo of Nelumbo either with or without the very short latent suspensor.—MEL. T. COOK, *Delaware Agricultural Experiment Station, Newark, Delaware.*

EXPLANATION OF PLATE VI

Abbreviations: *e* embryo; *t* tube nucleus; *n* egg; *s* synergids: *a* antipodals; *w* cross-wall.

FIGS. 1–7.—Diagrams of ovules, showing sac and nucellar tube: *fig. 1, Nymphaea advena; figs. 2, 3, Castalia odorata; figs. 4, 5, Castalia ampla; fig. 6,* Brasenia and Cabomba; *fig. 7, Nelumbo lutea.*

FIG. 8.—Two embryo sacs in axillary order; the one next to the micropyle is the larger.

FIG. 9.—Embryo sac of *Nelumbo lutea*, showing egg apparatus and endosperm nucleus.

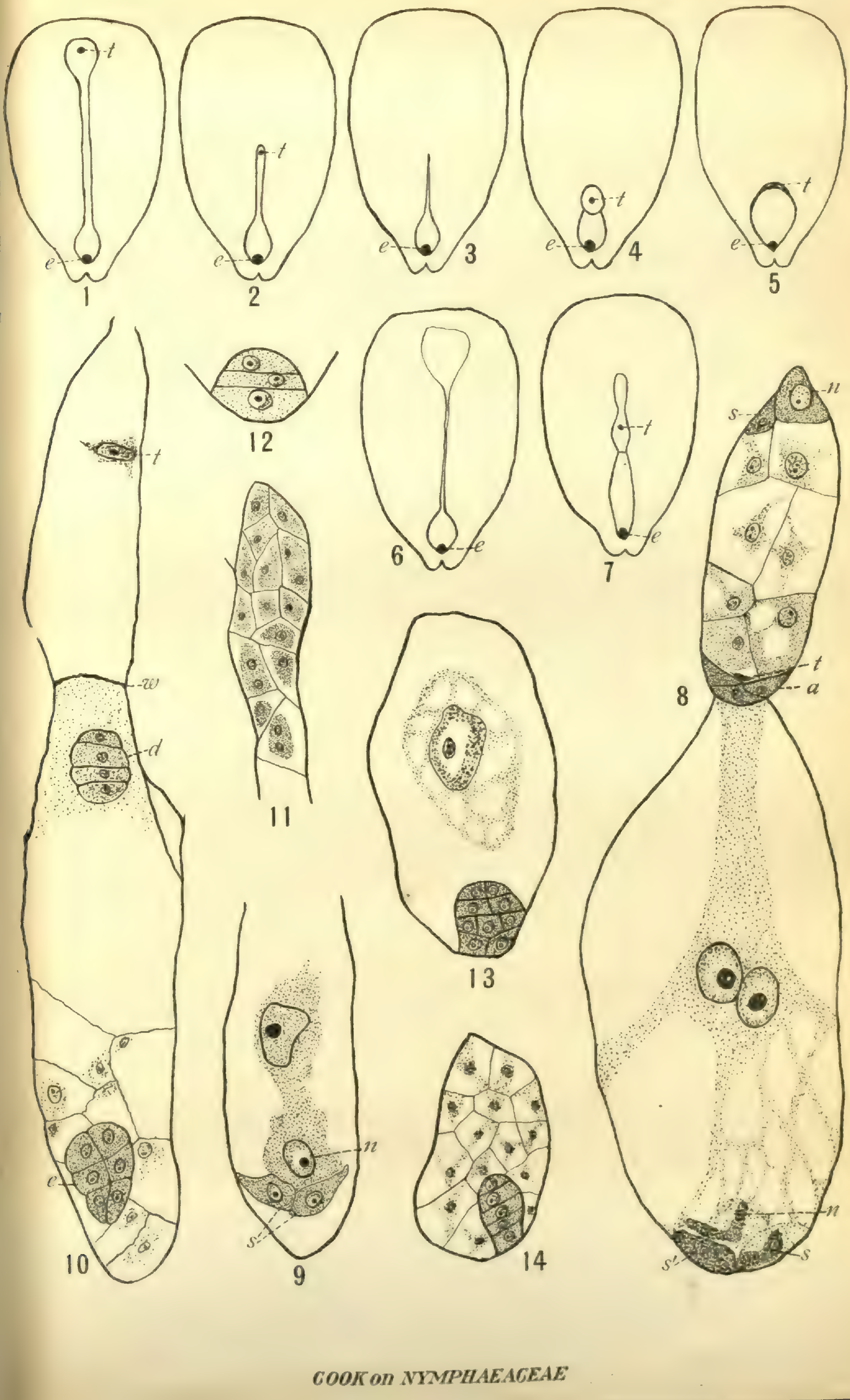
FIG. 10.—Embryo sac of *Nelumbo lutea*, showing embryo, cross-wall, and disentegrating tube nucleus.

FIG. 11.—Cells from the axial region of the ovule which may be the endosperm of unfertilized embryo sacs; from same section as *fig. 10*.

FIG. 12.—Embryo of *Nymphaea advena*.

FIG. 13.—Embryo of *Castalia odorata* and sac with the undivided endosperm nucleus; embryo without suspensor.

FIG. 14.—Embryo of *Castalia odorata* with suspensor.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CURRENT LITERATURE

BOOK REVIEWS

Mendelism.

A book by BATESON entitled *Mendel's principles of heredity*[1] has recently appeared from the Cambridge Press. The work is divided into two parts. The first part is a compilation and summary of all Mendelian work to date. The second part contains a biographical sketch of MENDEL and a translation of his two classic papers on hybridization in Pisum and Hieracium. The latter also appeared in the celebrated *Defense of Mendel*, published by the same author in 1902.

The body of the work is occupied with an account of the facts of Mendelian inheritance, as they have been accumulated since the work of MENDEL was brought to the attention of the scientific world at the beginning of the century; and a considerable proportion of this work is the result of the activities of BATESON and his students. The first three chapters contain the principles of Mendelian theory, as they have been developed and modified. The next five chapters deal with the phenomena of color heredity, indicating the prominent place the study of color characters occupies in Mendelian literature. The phenomena of "gametic coupling" and "spurious allelomorphism" are described and explanations suggested. Other chapters are concerned with heredity and sex; Mendelian inheritance in man; intermediates between varieties; Mendelian conceptions of the nature of units; the nature of segregation, reversion, variation, etc.; and the final chapter of part I is devoted to the practical application of Mendelian principles. The last chapter will prove useful to practical breeders of plants and animals, for even if there is not an *actual* segregation of characters, it is undeniably true that in many cases characters *behave as though* a segregation and recombination according to chance had taken place. There are certain cases, however, in which it is very difficult to suppose that a segregation takes place at the time of chromosome reduction. Such a case is that of two white races of sweet pea, one having long pollen and the other round. In the F_1 of this cross all the hybrids have long pollen, while if a segregation of characters had taken place during reduction, we should expect to find in each tetrad of pollen grains two long and two round.

The general aspects of Mendelian theory are but lightly touched upon in this work, since the author intends to deal with these in a separate volume. The remarks on every page, however, as well as the brief discussion of these topics leave no doubt as to the interpretation placed upon the phenomena described. It

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[1] BATESON, W., MENDEL'S principles of heredity. 8vo. pp. xvi+396. *pls. 6*, colored. *pls. 3*, half-tone portraits of Mendel. *figs. 37*. Cambridge: University Press; New York: G. P. Putnam's Sons. 1909. $3.50.

is a curious blindness to other facts of heredity which leads the author to the opinion that Mendelism probably represents the only type of inheritance which exists. Because characters sometimes behave as units does not exclude the occurrence of several other types of hereditary behavior, nor does the recognition of this fact belittle the facts of Mendelism. In the comparison of GALTON'S law of ancestral inheritance with the Mendelian ratios, the fact that GALTON'S law was designed for populations rather than for individuals seems to have been overlooked.

The method by which the process of segregation is visualized is very well exemplified in the following quotation (p. 56):

> Henceforth we have to penetrate behind the visible appearance of the individual, and endeavor to reconstruct first those processes of cell division which produced the germ-cells or gametes, distributing the characters or factors among them according to definite systems; and then the subsequent process of union of those gametes, pair by pair, in fertilization to form zygotes, each developing and manifesting in its development those properties of structure, instinct, and conduct conferred upon it by that particular complement of factors which its two original gametes contained.

Yet, fascinating as the theory appears, it must be remembered that it still remains an unproven hypothesis, to explain a characteristic method of hereditary behavior. The hypothesis has certainly proved useful, even though another explanation of the phenomena of segregation may ultimately be found necessary.

The book is attractively printed on a good quality of smooth paper, and appears to be exceptionally free from typographical errors. Its attractiveness is enhanced by three photographs of MENDEL and several colored plates, together with numerous illustrations and diagrams. A bibliography, and subject and author indices are found at the end of the volume. The work will be indispensable for reference by all students of heredity as a compendium of Mendelian phenomena.

A small volume entitled *Mendelism*, by R. C. PUNNETT, a collaborator with BATESON, was published in 1905 and passed through a second edition. An American edition[2] has just appeared, with a preface by GAYLORD WILSHIRE. It is a simple, clear account of Mendelian phenomena, and as such has doubtless done much to popularize Mendelism among general readers. The new edition also contains reprints of an article on "Applied heredity," which appeared in *Harper's Monthly Magazine*, and an article subtitled "Old Bottles," reprinted from *The New Quarterly*, which is chiefly a criticism of THOMSON'S volume on *Heredity*,[3] of the position taken by WALLACE and POULTON, and the attempt to minimize the importance of non-Mendelian types of inheritance. The paper is poor and the diagrams coarse, but the little book will doubtless serve its purpose as a cheap and popular presentation of Mendelism.—R. R. GATES.

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[2] PUNNETT, R. C., Mendelism. 12mo. pp. 109. New York: Wilshire Book Company, 200 William St. 1909. 50 cents.

[3] THOMSON, J. ARTHUR, Heredity. London. 1908.

The determination of sex

STRASBURGER'S latest work deals with the time of the determination of sex, apogamy, parthenogenesis, and the reduction division.[4]

The discussion in regard to the determination of sex is based largely upon the behavior of the spores of the dioecious liverwort Sphaerocarpus. Two spores of a tetrad give rise to male plants and the other two give rise to females. The conclusion is that the sex tendencies are separated during the two mitoses by which the four spores are formed from a mother cell. In the homosporous pteridophytes with monoecious prothallia, the expression of the sex tendency, as shown by the formation of antheridia and archegonia, does not take place so early, and in heterosporous pteridophytes all the spores of a tetrad, and even of a sporangium, produce plants of one sex. This is true also of spermatophytes, all of which have strictly dioecious gametophytes. STRASBURGER concludes, as had also CORRENS and NOLL, that the egg tends to produce females, and he believes that the mitoses in the pollen mother cell separate male tendencies of unequal vigor, so that, in dioecious plants, two microspores of a tetrad will give rise to sperms, which, in fertilizing the egg, are prepotent over the female tendency and so will produce males. The other two microspores of the tetrad will give rise to sperms which are not able to overcome the female tendency of the egg, and hence it will produce females. The hybrid obtained by pollinating *Fragaria virginica* with the pollen of *F. elatior* has been explained as a case of merogeny, but STRASBURGER found that fertilization occurs regularly, and that both male and female plants are produced. All the plants, however, resemble the male. This shows that the heritable characters of one of the nuclei which unite in fertilization can dominate the other. There is, as yet, no cytological evidence of the separation of sex-determining structures in plants.

Aside from a critical review of the literature, the discussion of apogamy is based principally upon an investigation of *Wikstroemia indica*, and 62 of the 88 figures illustrate critical stages in the life-history of this plant. As in other apogamous forms, the chromosome number is higher than in normal related species. In the pollen mother cells the mitoses differ from those of normal plants, and pollen tubes are never formed. The first mitosis in the megaspore mother cell shows some abnormalities. A wall is formed between the two daughter nuclei, but at the next mitosis, which usually occurs only in the lower cell, no wall is formed. An eight-nucleate embryo sac with a $2x$ egg is formed from the lower cell, and from this egg the $2x$ embryo is formed without fertilization. STRASBURGER regards this as a case of apogamy (*Eiapogamie*), and would reserve the term parthenogenesis for the development of an embryo from an egg with the reduced number of chromosomes. The reason for the discrimination is that he regards the $2x$ eggs as practically already fertilized.

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[4] STRASBURGER, EDUARD, Zeitpunkt der Bestimmung des Geschlechts, Apogamie, Parthenogenesis und Reduktionsteilung. Histologische Beitrage VII. 8vo. pp. xvi + 124. *pls. 1–3.* Jena: Gustav Fischer. 1909. *M.* 6.50.

In the heterotypic mitosis STRASBURGER interprets the double condition as a parallel conjugation and not as an early splitting, and in this parallel conjugation he finds the explanation of the reduction from the $2x$ to the x number of chromosomes. *Galtonia candicans* furnishes particularly strong evidence that there is a pairing rather than a splitting.

STRASBURGER was one of the first to claim that the nucleus is the physical basis of heredity. Since it has been urged that other structures are concerned, the sperm nuclei of Lilium were carefully reexamined, and it was found that only the nucleus, with no trace of cytoplasm from the pollen tube, enters the egg.

The book closes with some interesting suggestions in regard to the origin and development of the nucleus: The original protoplasm had no nuclei, all its parts being capable of both formative and nutritive functions. Then there was a gradual separation of formative and nutritive parts, and the formative parts were the first differentiated carriers of hereditary qualities. At first they were scattered in the cytoplasm, but later became grouped, as in the Cyanophyceae. Next the nucleus would be marked off from the cytoplasm by a membrane. Simple constriction might suffice for the division of such a nucleus, but as the difference between hereditary units became so great that each unit carried only one quality, a more exact division would become necessary. The units would become arranged longitudinally in a thread, where they would undergo a doubling, and the longitudinal division of the thread would separate the product of that doubling. The complete resemblance between the mitoses of higher plants and animals makes this sequence very probable.

The book touches upon almost every phase of cytological investigation and consequently only a few of its more important features can be mentioned in a review.—CHARLES J. CHAMBERLAIN.

Biology of chlorophyll

Starting from the complementary colors of marine algae, an accepted adaptation to the light at varying depths, as suggested by ENGLEMANN (1883), and the complementary tints attained promptly by certain Oscillatoriae, when exposed to light of differing hues, as shown by GAIDUKOW (1902), STAHL proposes to inquire why plants are green and not some other color, and whether the green of land and water plants is not an adaptation to the composition of sunlight, modified by its passage through the atmosphere. Citing the results of physical investigations to show that in diffuse light the blue and violet rays prevail, and in direct sunlight the red and yellow, he claims that an unrecognized relation exists between these facts and the selective absorption of the chlorophyll. The yellow component serves to absorb the blue-violet; the green component the red-yellow. The yellow pigment is complementary to the blue of the sky, and the green to the red-yellow rays which pass through an atmosphere whose haziness becomes evident to our

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[5] STAHL, ERNST, Zur Biologie des Chlorophylls: Laubfarbe und Himmelslicht Vergilbung und Etiolement. 8vo. pp. vi + 154. *pl.* 1. Jena: Gustav Fischer. 1909. *M* 4.

eyes when the sun is low. As the red Florideae are colored in complement to the green-blue rays least weakened by traversing sea water, so the green and yellow of chlorophyll are complementary to the red and blue that traverse the atmospheric sea. It thus appears that the yellowish green of vegetation is really an adaptation to the composition of diffuse light.

Various lines of reflection, more or less closely related to this idea, are elaborated, such as, Regulation of the absorption of the sun's rays; Variable content of chlorophyll in nutritive organs; Biology of non-green algae; Etiolation, autumnal yellowing, and their biological significance.

The book is almost purely philosophical, only a few experiments, and these avowedly superficial, being recorded. Of course the general thesis does not touch the fundamental reason for plant coloration, and it is doubtful whether there is any logical gain in this mode of looking at the facts. To give it validity, it must be shown that the absorption of other rays by different pigments (and consequently a different colored vegetation) would be nutritively inefficient. Considering the fact that most plants get far more energy than they can use at ordinary temperatures, by reason of the inadequate supply of CO_2, it would surely be difficult to show this.—C. R. B.

MINOR NOTICES

Bacterial classification.—JENSEN has attacked the difficult problem of bacterial classification with a view to making a "natural system," that is, one which will express relationship by descent.[6] Most attempts to classify bacteria have been based first upon form or upon mode of cell division, and then upon physiologic characters. This method has led to many anomalies. For example, in one sharply defined family, the red sulfur bacteria, there are found rod, spiral, and round forms; and in the case of certain species of Crenothrix and Azotobacter, division occurs first in one, later in three planes. JENSEN argues that bacteria with a single flagellum, or with tufts of polar flagella, are closely allied, but that these are sharply differentiated in fermentative as well as other reactions from those having flagella distributed over the cell-body. Hence he divides the whole bacterial kingdom into two orders: (1) Cephalotrichinae and (2) Peritrichinae. In the former he groups seven families, in the latter four families. For genera he selects, as a means of discrimination, the oxidizing, reducing, fermentative, and other chemical processes of metabolism, as shown by the work of himself, WINOGRADSKY, BEIJERINCK, KASERER, BURRI and STÜTZER, MOLISCH, OMELIANSKI, BÜCHNER, and others. JENSEN regards the genus Methanomonas as the origin of all organic life, since this autotrophic, monotrichial, rodlike form builds up its cell substance in the simplest manner possible, without requiring either organic carbon, organic nitrogen, or outside energy such as light or heat. On the contrary, a certain

[6] JENSEN, O., Die Hauptlinien des natürlichen Bakteriensystems nebst einer Uebersicht der Gärungsphänomena. 8vo. pp. 42. *fig.* 1. Jena: Gustav Fischer. 1909. *M* 1.

amount of energy is actually set free during the metabolic process of *Methanomonas methanicus*. From this genus evolution progresses in four directions, toward the Protozoa, the Eumycetes, the Cyanophyceae, and toward the putrefying and pathogenic forms. The tree of descent thus outlined is ingenious, and species seem to fall into places of natural relationship. This, however, may only mean that generic distinctions are founded on well-established biological properties, for it is probable that bacteriologists will hesitate to accept a primary division into orders on a basis of the distribution of flagella.—MARY HEFFERAN.

Botanical directory.—A third edition of DÖRFLER'S *Adressbuch* was issued some months ago.[7] It is needless to commend this useful volume to those who know the earlier editions; but we do a real service to any who do not by directing their attention to the greatly improved third edition. The list of botanists with their addresses and specialties is of course the main feature; but very useful also are the lists of botanical periodicals and societies.

If any fault is to be found with the directory it is because it includes too much rather than too little. For example, we would have spared gladly the 270 pages of so-called "bibliography"—really a trade catalogue; but perhaps the author, who is also the publisher, could not afford (financially) to leave it out.

Further, there are too many names included. How to sift the real botanists from the tradesmen and notoriety seekers is a difficult problem; for some of our best-known but heedless botanists have not responded to the circulars of the editor, and many of the sham botanists have. Judging by the United States list the numbers might be reduced 50 per cent., for everybody knows that there are not in this country half of the 1800 listed who ought to be in a botanists' directory. We may suggest to the author the possibility of securing the cooperation of one or more editors in each country to purge the list. Why would not the issuance of this directory as an annual be a good work for the *Association internationale des botanistes?* Certainly it cannot be much of a money-making venture, and no doubt the editor would be glad of support. He is certainly entitled to great credit for the labor he has expended and the risk he has assumed in issuing this indispensable volume.—C. R. B.

Mechanics of plants.—Those who desire a compact and authoritative statement of SCHWENDENER'S present views on the mechanics of plants, as set forth in a course of special lectures given regularly for advanced students in the summer semester at Berlin, will find them in a thin volume[8] recently published by Profes-

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[7] DÖRFLER, J., Botaniker-Adressbuch. Sammlung von Namen und Adressen der lebenden Botaniker aller Länder, der botanischen Gärten und der die Botanik pflegenden Instituten, Gessellschaften und periodischen Publikationen. Dritte neu bearbeitete und vermehrte Auflage. 8vo. pp. viii + 450. Wien: The author (III, Barichgasse 36). 1909. $3.35.

[8] HOLTERMANN, CARL, SCHWENDENER'S Vorlesungen über mechanische Probleme der Botanik, gehalten an der Universität Berlin. 8vo. pp. vi + 134. With portrait of SCHWENDENER and *90 figs.* Leipzig: Wilhelm Englemann. 1909. *M* 3.60.

sor Dr. HOLTERMANN, a docent in the botanical institute. The work does not profess to be a verbatim report of SCHWENDENER's lectures (which he himself could not make ready for the press), but is an expression of his views, based upon his lectures, published works, and personal communications; and moreover, the book has had his revision. The contents must suffice to show the character of the work: The mechanical system; Theory of phyllotaxy; Ascent of sap; Stomata; Twining; Tension of cortex; Distortion of pith rays by excentric growth; Apparatus for gliding; Turgor movements; Hygroscopic curvatures and torsion.

To have these topics treated clearly and tersely is extremely useful, and Dr. HOLTERMANN's service will be appreciated.—C. R. B.

Pflanzenfamilien.—With the exception of a supplement to the section on lichens, which is in preparation, this monumental work[9] has come to an end with *Lieferung 235*, concluding BROTHERUS' exposition of the mosses. It has been in course of publication since 1887, the last part being issued in March, 1909. The high appreciation of botanists all over the world must be the reward of the distinguished senior editor, ENGLER, whose great plan has been so successfully executed. To the publisher, WILHELM ENGELMANN, also, are due congratulations for his courage in undertaking so huge a work, whose commercial success must have been problematic at the outset, and for his efficiency in the details of publication.—C. R. B.

NOTES FOR STUDENTS

The perithecium of Ascomycetes.—The whole of Vol. X of *Le Botaniste* is devoted to an elaborate paper by DANGEARD[10] on the origin of the perithecium in the Ascomycetes. Pages 1–26 are devoted to a general discussion of the subject, while pp. 27–385 are given over to the description of the individual plants studied. The writer is committed thoroughly to a belief in the autonomous nature of the fungi as a group, and in the derivation of the higher forms from phycomycetous ancestors. He postulates at the outset that the ascus is an organ of the same nature throughout the group; that it had a common origin; and that the slight variations which appear are the results of adaptation. He is confronted with four diverse opinions regarding the nature of the ascus: (1) that it is a modified sporangium; (2) that it is a sporocarp or part of a sporocarp; (3) that it is a mother cell presenting special characters; and (4) that it is a sporogonium. Each one of these theories is then discussed. The first, which is that of BREFELD, regards the ascus as a sporangium in which the form has become fixed and the number of spores definite. This is held to be untenable, since it implies the complete absence

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[9] ENGLER UND PRANTL, Die natürlichen Pflanzenfamilien, etc. 234 und 235 Lieferung. Brachytheciaceae (Schluss), Hypnodendraceae. Nachträge und Verbesserungen. Von V. F. BROTHERUS. Teil I. Abt. 3. pp. 1153–1246. Leipzig: Wilhelm Engelmann. 1909. *M* 3.

[10] DANGEARD, P. A., L'origine du périthèce chez les Ascomycètes. Le Botaniste **10**:1–385. *pls. 1–91.* 1907.

of sexuality in the Ascomycetes, and that the phycomycetous ancestors have transmitted to the higher fungi only the asexual stage. Since asexual conidiophores are numerous in the Ascomycetes, the primitive aerial sporangium must have developed along two different lines, giving rise on the one hand to the ascus and on the other to the conidiophore.

According to the second theory, that of DE BARY, the ascus is either the whole or part of a multicellular structure, the sporocarp, which is the product of the fertilization of an archicarp. The latter is a cell capable of being fertilized, and finds its homologue in the receiving gamete of the alga, bryophyte, or fern. The sporocarp corresponds, therefore, to the oospore or zygospore of the green alga, the sporogonium of the bryophyte, or the leafy plant of the fern. In other words, it is the sporophyte. If all the Ascomycetes developed their asci in the manner of *Dipodascus* or *Eremascus*, this conception of the organ as a sporogonium or sporophyte would hold true, since in these plants the ascus arises directly from the fertilized egg. But DE BARY believed that in the greater number of the Ascomycetes the archicarp gives rise to the whole perithecium, so that the ascus in these cases is not an entire sporogonium but only a part of it, and he regarded it as the equivalent of the spore mother cells in the bryophyte or fern. But it would be remarkable if an organ of such uniform structure should correspond in some cases to a whole sporogonium, and in others to a mother cell only; and DE BARY was in error in thinking that it could be either one or the other according to circumstances.

As to the third theory, the author remarks that, however strange it may seem to homologize asci with spore mother cells, it must be admitted that a certain analogy exists in the limited number of divisions in the ascus to form the spores. If the whole perithecium really arose from the fertilization of an egg, no serious objection could be urged against this homology. But the fruiting body of the Ascomycetes has a mixed origin, since part of the envelope is formed from filaments of the gametophyte. The following additional facts are regarded as antagonistic to this view: (1) In the small number of genera in which the gametangia are still functional, the egg gives rise directly to the ascus, as in *Dipodascus;* (2) in the other genera the gametangia are no longer functional, and the perithecium does not develop from the egg; (3) the formation of the ascus is always preceded by a fusion of nuclei, and no case is known in which the formation of mother cells is preceded or accompanied by a phenomenon of this kind; (4) in the lower siphonaceous fungi, from which the Ascomycetes have arisen, there is no organ which corresponds to mother cells, but one finds on the contrary the sporogonium (oospore, etc.), which is evidently the ancestor of the ascus.

Much discussion is devoted to HARPER'S contention that the fusion of nuclei which precedes the formation of the ascus is without sexual significance. Three points are considered in this connection: (1) the binucleate structure of the ascus; (2) the fusion of the two nuclei into one; (3) the reduction in the number of chromosomes. Against HARPER'S contention, that the binucleate condition of the ascogenous cells is the result of the stimulus of excessive nutriment, it is urged

that the rôle of nutrition is secondary in nuclear division; that more importance should be attached to the proportion of carbon compounds in the protoplasm and to tendencies transmitted from ancestral forms; and that the constant occurrence of two nuclei has more than a nutritive significance. Their fusion must be regarded as of a sexual nature because of the fusion of two energids into one; because the result of the fusion is a sporogonium as in the case of the ordinary egg; because nuclear fusion necessitates a reduction of chromosomes; and because the formation of gametes on the gametophore recalls exactly the transformation of sporangia into conidiophores and has the same cause.

The author believes that the nuclear fusion in asci and basidia really represents the sexual reproduction in the higher fungi, and that the fourth theory mentioned is the true one, viz., that the ascus is a true sporogonium in all species and is therefore sporophytic in its nature. He believes that the primitive ancestral phycomycete had a greater or less resemblance to *Myzocytium vermicolum*. It possessed a thallus bearing sporangia, which, in his opinion, represented the sporophytic stage. The sporangia were very susceptible to external conditions. This was followed by a thallus bearing gametangia, the gametophyte; the fused gametes gave rise to the fertilized egg, which on germination produced a new sporangium or *sporogonium* with the characters of an ordinary sporangium, but with the doubled nuclei. This sporangium was but slightly susceptible to external conditions. The author believes that this point should be strongly insisted upon, that the ancestral type possessed two sorts of sporangia: the one responsive to environment has given rise, under the influence of aerial life, to the various types of conidiophores met with in the Ascomycetes; the other, of sexual origin, has been modified but little and has given rise to the ascus.

The author proposes a new classification of the Ascomycetes, based on the reproductive organs, in which the whole group is divided into two, the *Gamétangiées* and *Gamétophorées*. The first possesses functional gametangia and includes *Dipodascus* and *Eremascus*. In the second the gametangia, if present, are no longer functional, and their place is taken by gametophores.

About forty-five different types of Ascomycetes are described and figured in the major portion of the paper. One cannot but marvel that a single investigator could have found time to study all these forms in detail and with thoroughness. A most confusing use of terms makes the whole discussion difficult to follow.—ELIAS J. DURAND.

Algal-animal symbiosis.—KEEBLE,[11] continuing his interesting experimental investigation of the associations of unicellular algae with the low animals occurring on the larger seaweeds living between and just below the tide-limits on the north coast of France, now reports the occurrence of a unicellular brown alga in *Convoluta paradoxa*, a tubellarian. He had previously found a green Chlamydomonas-

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[11] KEEBLE, F., The yellow-brown cells of *Convoluta paradoxa*. Quart. Journ. Micros. Sci. **52**:431–479. 1908.

like alga in another species of Convoluta.[12] The color of the animal is due in part to orange-red glands in the superficial tissues, and in part to yellow-brown algal cells in the deeper tissues as well. The animals appear always to contain these yellow-brown cells, and in attempted cultures of the animals from the eggs, under conditions precluding infection by algae, the animals fail to develop. Furthermore, when older animals, already containing numerous yellow-brown cells, are kept in the dark, the yellow-brown cells disappear, apparently being gradually reduced to indigestible granular remnants. Such animals, again lighted, fail to develop further unless reinfected; then, however, they grow rapidly as soon as the new algae have sufficiently multiplied.

These facts indicate a dependence on the part of the animal upon its algal associates which may well be called parasitism, a degree of parasitism carried to the extreme of consuming the algae only when starvation impends, and under normal conditions falling far short of such destruction. In describing such an association as this, in which one animal becomes parasitic upon many much smaller algae inclosed within its own tissues, our usual vocabulary suffers strain, for words have to be used with altered meanings. Thus we should naturally speak of the animal being infected by the algae, and wonder how it is accomplished; but shall we speak of the infection of a parasite by its host? The relation of host and parasite in these tubellarians is like that of alga and fungus in lichens, the parasite incloses, lodges, and in time of hunger may ultimately devour its host. These convolutas secure their hosts by ingesting them with other food. The animal feeds voraciously on a varied diet. Apparently in these animals, as in many others, digestion of great quantities of food is less complete than when only smaller quantities are taken at once. The brown algae, if ingested in small quantity, are all digested and destroyed, but if the quantity of food is large, some cells escape digestion, entering the tissue of the animal and in a way becoming a part of it.

KEEBLE sees in the photosynthetic activity of these algal cells their value to the animals. The unicellular brown algae contain chromatophores in which there are two pigments: a yellow, quickly soluble in hot 90 per cent. alcohol and decomposed by hot water (fresh?), and a green, which resembles chlorophyll. The products of photosynthesis KEEBLE says he has not been able to determine, but he regards the fat globules in the cells as the form in which the photosynthate is temporarily stored. These fat globules he has seen migrating from the algal cells into the animal tissues, and their number is directly proportioned to the state of nutrition of the two organisms, being abundant in well-lighted animals immediately after being brought into the laboratory, decreasing under the somewhat unnatural conditions of the laboratory, and disappearing entirely if the cultures are placed in filtered (that is, food-free) sea water in the dark. The synthesis of food from CO_2 and H_2O within its own body, if unaccompanied by deleterious products, is of obvious advantage to an animal. Yet the advantage seems to be less in *Convoluta paradoxa* than the earlier studied *C. roscoffensis*. In the latter, the

[12] See review in BOT. GAZETTE 46:68. 1908.

animal presently ceases to take food from without, and is nourished entirely by its endophytic algae; in the former, the animal continues to feed throughout its lifetime. For some reason, the parasitism is less complete.

The benefit to an animal in association with a plant is much more obvious than the benefit accruing to the plant. It is customary among naturalists to conceive of every structure, every act, every situation, as useful or advantageous to the organism, or as having been useful or advantageous in the past. Many cases might be cited which stultify such a position, yet KEEBLE seeks to find some value to the independent alga in its association with a dependent animal, and believes that it consists in a "solution of the nitrogen problem—a successful method of obtaining large supplies of nitrogen." This is plausible, and adding a usable nitrogen compound, such as uric acid or potassium nitrate, in due proportions to the food-free filtered water of laboratory cultures, prolongs the life and prosperity of plant and animal alike. But as KEEBLE plainly indicates, this profits individuals, not the species. Ingested algae of a certain sort, escaping digestion and manured with animal wastes, multiply in the tissues of this small animal; they grow and prosper; but beyond the body of the individual animal they do not appear to spread. They do not infect the eggs; they do not escape from the body of convoluta before or after its death; they die with it. Each convoluta larva is separately and freshly "infected" by some of its food which it fails to digest. But although KEEBLE has failed to find the alga in its free form, he believes that it has one, and that the species is continued by those individuals which escape the convolutas. Indeed this belief is inevitable, if the ingested and surviving endophytes produce no successors.

I can do no better than quote KEEBLE's own vigorous summary. "The interpretation of the relation between yellow-brown cell and animal depends on the point of view: From that of the animal it is obligate parasitism. From that of the species 'infecting organism,' it is an insignificant episode, involving the loss of that, probably small, proportion of its numbers which are ingested. From that of the individual ingested yellow-brown cell, it is a solution of the nitrogen problem—a successful method of obtaining large supplies of nitrogen."

Obviously, then, to the species of alga there is no use in the association; to certain individuals, which apparently produce no successors, there may be some advantage. Even if there were no other, I suppose some persons would claim an advantage for the algae which escape digestion and survive in the body of the animal, on the hypothesis that life is better than death!

KEEBLE has not yet succeeded in finding the yellow-brown cells in the free state, in cultivating them free from the animal body, or in identifying them with any now known algae; yet he has no doubt of their being algae. There is little reason either to question his opinion or to doubt his ultimate success in cultivating and identifying them.

The theoretical value and interest of this paper is greatly enhanced by the skill and care of the author as experimenter, writer, and draftsman.—G. J. PEIRCE.

Inheritance of albinism.—BAUR[13] has investigated the cause and inheritance of the white-margined condition found in the leaves of some of the common varieties of pelargonium and other forms. The white region of such leaves is composed of cells having colorless instead of green chromatophores, and the periphery, including the growing points, of such plants is found to be composed of 2 or 3 rows of colorless cells. Green cells are ordinarily descended from green, and white from white.

When reproduced by seed the white-margined forms, since their sexual cells are from the peripheral white region, produce pure white offspring, which, having no chlorophyll, are incapable of growth. The occasional white branches produce pure white offspring; and the green branches produce pure green offspring. From the union of a "white" with a "green" sexual cell three kinds of plants are produced: pure green, which breed true; pure white; and green-white mosaics. It is probable that all belong to the last class, the pure white and green being extreme cases in which one type of cell has displaced the other at an early stage.

In the hybrids which show a mosaic of white and green cells in their cotyledons, if the growing point is situated in a green part, then the plant produces only green parts thereafter; if in a white part, only white will be produced; if on the border between white and green, a *sectorial chimera* will be produced, one side of which will bear only green leaves and the other side only white. The production of a chimera of this sort by grafting was recently described by WINKLER[14] in Solanum.

If the growing point is periclinally divided into green and white cells, then the leaves appearing will have the characteristic white margins. In other words, the white-margined varieties of pelargonium are *periclinal chimeras*, the white and green cells of which are both genetically descended from cells of their own sort.

Islands of white tissue surrounded by green in the young seedlings show that the white cell may originate repeatedly from green cells. BAUR'S hypothesis of the origin of these types of cells is that the fertilized egg contains both types of chromatophore, which are distributed chance-wise in subsequent cell divisions. If a daughter cell thus comes to have only colorless chromatophores, then all its descendants will be colorless.

Whether the chromatophores are transmitted through the egg alone, as generally believed, or also through the male cell, BAUR hopes to determine. If the former were the case then a "green" male nucleus, for example, would necessarily be able by its influence to cause the development of green in certain of the chromatophores in a "white" egg. A cytological study, to determine the origin and development of chromatophores in the embryo, and the form in which they may be present in the sexual cells, is greatly needed.—R. R. GATES.

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[13] BAUR, EDWIN, Das Wesen und die Erblichkeitsverhältnisse der "Varietates albomarginatae Hort." von *Pelargonium zonale*. Zeits. Abst. u. Vererbungslehre **1**:330–351. *figs.* 20. 1909.

[14] WINKLER, HANS, Ueber Pfropfbastarde und pflanzliche Chimären. Ber. Deutsch. Bot. Gesells. **25**:568–576. *figs.* 3. 1907. See BOT. GAZETTE **47**:84. 1909.

Centrosomes in Stypocaulon.—Since STRASBURGER and SWINGLE'S studies, Stypocaulon, one of the brown algae which bears a conspicuous apical cell, has been considered as showing typical centrosomes in thallophytes. According to their account, the centrosome, single at first, divides into two centers, which become surrounded by astral radiations. SWINGLE also described the kinoplasm as clearly distinct from the trophoplasm, the former being the only part of the cytoplasm concerned in the formation of achromatic spindles.

In GRÉGOIRE'S laboratory, ESCOYEZ[15] has recently studied the origin of chromosomes and spindles in the nucleus of the apical cell of this plant, and his conclusion is quite different from that of the authors mentioned above. The résumé of ESCOYEZ'S results is as follows: (1) In an apical cell, the chromosomes are formed at the expense of a certain part of the nuclear network, the rest and greater part of which remains unused. (2) A nucleolus does not take part in forming the chromosomes; possibly it may furnish chromatin substance to the forming chromosomes, since it becomes disorganized during the prophase. (3) In the telophase, the chromosomes reconstitute the chromatin network in the usual way; a nucleolus then appears, but not by the confluence of chromosomes. (4) Spindles are probably of cytoplasmic origin. (5) The two asters, whose appearance marks the beginning of prophase, do not result from division of a single aster, but they are formed independently, one after the other. (6) It s impossible to distinguish two elements, such as the kinoplasm and trophoplasm, as interpreted by STRASBURGER. Very likely the central bodies which lie in the center of asters are not true centrosomes in BOVERI'S sense. They give no evidence of having the nature of the true centrosomes, but represent only cytoplasmic microsomes.—SHIGÉO YAMANOUCHI.

Temperature of insolated leaves.—SMITH, using the new slender thermojunctions of BLACKMAN and MATTHAEI, has determined the internal temperature attained by leaves placed normal to sunlight at Peradeniya.[16] He finds little difference between thin and fleshy leaves in the final temperature attained, which is some 15° C. above that of the air (25–28°), but the latter are less rapidly heated. In the shade the same leaves are 1°.5 below to 4° above the air temperature. He estimates the cooling due to transpiration at 2°.5, which is incredibly low, considering the energy required to evaporate the water transpired. Breezes, even the most moderate, are the more efficient cooling agents, reducing the temperature by 2°–10°. Red leaves were found to attain higher temperatures than a white or pale leaf of like texture and thickness.

The second part of the paper has little connection with the first. In it SMITH records observations to show that the new growth of a large number of trees

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[15] ESCOYEZ, E., Caryocinèse, centrosome et kinoplasme dans le *Stypocaulon scoparium*. La Cellule **25**: 181–201. *pl. 1*. 1908.

[16] SMITH, A. M., On the internal temperature of leaves (we omit the rest of a five-line title). Annals Roy. Bot. Gard. Peradeniya **4**: 229–298. 1909.

at Peradeniya occurs in the driest part of the year (all of which is very wet). To account for this, he suggests that it is only in the drier period that the transpiration stream can supply enough salts! Then on this assumption he erects another: "If this be the case, the higher internal temperature attained by the coloration of young leaves would promote the some object, viz., the increase of the transpiration stream." All of which is an excellent example of spoiling good observations by bad logic.—C. R. B.

Sex in dioecious plants.—A study of the two mitoses by which the microspores are formed from the mother cell in *Acer negundo* has led DARLING[17] to interpretations and conclusions about which there may be considerable difference of opinion. He finds that all the chromatin of the resting nucleus of the microspore mother cell is contained in the nucleolus. Chromatin from the nucleolus diffuses upon the linin and in this way there is built up a spirem which segments into eight chromosomes. Later, five more chromosomes are formed from the nucleolus, so that, all together, there are thirteen chromosomes formed in these two ways. After the second mitosis, two of the daughter nuclei differ from the other two in containing one more chromatin mass, but when the resting stage is reached, the four nuclei look alike.

The writer believes he has found something somewhat analogous to the maturation mitoses in some insects, and that the peculiarities in *Acer negundo* have some connection with the determination of sex.

The fact that the division of the chromosomes at the second mitosis could not be determined with certainty would indicate that the technic was hardly sufficient to establish the claim that the eight and five chromosomes originate differently. The problem, however, is important and the presentation of results suggestive.—CHARLES J. CHAMBERLAIN.

Chlorophyll of seeds.—MONTEVERDE and LUBIMENKO, working independently, have arrived at the same conclusion regarding the green pigment of the seeds of thirty-eight Cucurbitaceae, viz., that it is not chlorophyll, but that it resembles the protochlorophyll of etiolated leaves.[18] Yet neither in the living nor the dead hulls does it go over, under the influence of light, into chlorophyll. It appears rather late in the development of the seed, in chromatophores which are indistinguishable from chloroplasts and may even contain chlorophyll also. Its absorption spectrum differs in certain details from that of living green leaves. They propose to call this new pigment chlorophyllogen, retaining the name protochlorophyll for the optically altered chlorophyllogen which one can observe in dead tissues and neutral solutions. This chlorophyllogen becomes transformed into chlorophyll under the influence of light plus some other yet unknown factor

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[17] DARLING, CHESTER ARTHUR, Sex in dioecious plants. Bull. Torr. Bot. Club **36**:177–199. *pls. 12–14.* 1909.

[18] MONTEVERDE, N., UND LUBIMENKO, W., Ueber den grünen Farbstoff der inneren Samenhülle einiger Cucurbitaceen und dessen Beziehung zum Chlorophyll. Bull. Jard. Imp. Bot. St. Petersbourg **9**: 27–44. 1909. (Russian: German résumé.)

(possibly an enzyme produced only in light), which is not operative in cucurbitaceous seeds, but is active in etiolated leaves.

Perhaps other so-called chlorophyll originating in the dark will prove to be only this forerunner of chlorophyll. The authors will continue their further researches together. Out of 800 species in 110 families observed, they have found chlorophyllogen in representatives of 18 families.—C. R. B.

Nucleoli in Marsilia.—To prove whether or not there is any transfer of chromatin substance into nucleoli and vice versa, BERGHS[19] has studied vegetative mitosis in the root and prothallium of *Marsilia macra* and *M. Drummondii*. A peculiar condition of the nucleolus in Marsilia was described two years ago by STRASBURGER. The resting nucleus in these species generally contains a single conspicuous nucleolus, which always takes stains deeply, and at a certain stage almost all of the stained substances are found only in the nucleolus. BERGHS, after following the whole processes of vegetative mitosis in the meristem of the root and in the young prothallium, gives the following results. The nucleolus is achromatophile at the moment of its appearance, and afterward becomes more and more chromatophile; at the same time the chromatin network loses its chromatin and decreases in size. The chromatin network in the resting nucleus certainly does not contain the total substance of the definite chromosomes, and in the prophase the nucleolus loses chromatin matter during the formation of chromosomes. The fact that the nucleolus is formed as an achromatic substratum and becomes impregnated with chromatin material during the resting stage, the author believes, indicates a transfer of chromatin material between the nucleolus and chromosomes.—SHIGÉO YAMANOUCHI.

Development of Aeginetia.—The morphology and anatomy of *Aeginetia indica* were described by KUSANO a few years ago, but he found nothing peculiar which would distinguish it from Orobanche. But it does show interesting features in its germination and early growth, which he describes in a recent paper.[20] The short-lived seeds germinate only under stimulation by some substance or substances arising from the roots of vascular plants, and the development of the seedling takes place only on certain species of monocotyledons. The first sign of germination is the appearance of large globular cells at the radicular end of the embryo, from several of which develop hairs, sometimes a millimeter in length, that protrude in all directions. When they come into contact with the host root, they attach themselves (by slight insertion or cement?), and then coil irregularly, thus drawing the embryo close to the host. These tendril-like hairs KUSANO calls hair-tendrils. If the host be suitable the seedling develops a tubercular body, from which arises the primary haustorium. This secures nutriment from the host, making possible later the development of a stem and root system, which arise much as in Orobanche.

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[19] BERGHS, J., Les cinèses somatiques dans le Marsilia. La cellule **25**:73–84. *pl. 1.* 1908.

[20] KUSANO, S., Further studies on *Aeginetia indica*. Bull. Coll. Agric. Tokyo Imp. Univ. **8**:1–20 (?). *pl. 7.* 1908.

The stimulus to this development is quite other than that for the hair-tendrils. The latter, according to the author, have not been discovered heretofore in any seed plant.—C. R. B.

Phylogeny of the embryo -sac and double fertilization.—PORSCH [21] attempts to explain the usual eight-nucleate embryo sac of the angiosperms as an extreme reduction from the gymnosperm type, consisting of two reduced archegonia, the egg apparatus and the upper polar nucleus representing one archegonium, and the three antipodals with the lower polar nucleus representing the other. The egg apparatus is regarded as made up of an egg, two neck cells (the synergids), and a ventral canal cell (the upper polar nucleus). The antipodal complex is interpreted in the same way. Then double fertilization might be an attempt to fertilize both archegonia.

While a combination of all the features presented by gymnosperm and angiosperm gametophytes can be arranged in such a sequence, the reviewer does not believe that there is anything phylogenetic in it. Though the angiosperm embryo sac has doubtless come through stages in which there was a tissue with archegonia, we do not believe that any part of the archegonium, except the egg itself, has been retained.—CHARLES J. CHAMBERLAIN.

Chemotaxis of Lycopodium sperms.—BRUCHMANN'S discovery that the sperms of Lycopodium are sensitive to citric acid and its salts is a disturbing factor in SHIBATA'S generalization that the chemotactic sensitiveness of the three great pteridophyte lines toward malic acid approves the theory of their monophyletic origin. Yet BRUCHMANN, apparently not sensing the humor of this idea, gravely softens the blow by suggesting that this aberrant behavior of the Lycopodium sperms does not place the lycopods outside this line of descent, but is to be explained by the saprophytic character of the odd gametophyte![22] He shows that malic acid is not responded to, and that saccharose, glucose, lactose, albumin and other proteins, acetic, oxalic, formic, and butyric acids are likewise ineffective. But to citric acid 1:1,000,000, and to its alkali salts in 1:100,000, the sperms respond positively, the reaction becoming negative at 1:1000 and 1:100 respectively. Acids and alkalies proved repulsive, as in the case of other sperms. The Weber law was found to be valid, with the ratio 1:30–40.—C. R. B.

Geotropic perception.—NEWCOMBE, after criticizing again the weakness of the experiments for the localization of geoperception, has used the old decapitation method for demonstrating that in some species as much as 4 or 5^{mm} of the root tip is capable of perceiving the gravity stimulus.[23] He is unable to relate the

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[21] PORSCH, OTTO, Versuch einer phylogenetischen Erklärung des Embryosackes und der doppelten Befruchtung der Angiospermen. Vortrag gehalten auf der 79. Versammlung deutscher Naturforscher und Aertzte in Dresden am 16. Sept. 1907. pp. 1–49. 14 text figures.

[22] BRUCHMANN, H., Von der Chemotaxis der Lycopodium-Spermatozoiden. Flora **99**:193–202. 1909.

[23] NEWCOMBE, F. W., Gravitation sensitiveness not confined to apex of root. Beih. Bot. Centralbl. **24**:96–110. *pl. 3. figs. 6.* 1908.

extent of this region to the length of the elongating zone. PICCARD'S method of 1904, which has lately been justified by HABERLANDT,[24] is considered by NEWCOMBE as "too precarious to be satisfactory." All the phenomena, he concludes, "accord equally well with the hypothesis of the extension of the sensitiveness through the elongating zone, but diminishing from the apex backward; or . . . . of a more equable sensitiveness through the elongating zone, and a stronger autotropism in the posterior than in the anterior part." We should much prefer the former hypothesis; if for no other reason, because it is unfortunate to postulate "autotropism" when it can be avoided.—C. R. B.

Development of Marchantia.—Because no consecutive account of the development of the sexual organs and sporogonium of Marchantia, complete in itself, has been published by one author, DURAND, while preparing slides for class use, has published an account of the development illustrated with a close series of figures.[25] The account contributes little which is new to students making a critical study of this form. For the first time, although it has been illustrated by CURTIS, formal attention is called to the familiar "mushroom anchor" foot. One striking feature in the development of the sporophyte has been overlooked: the sterile plate of cells at the apex of the capsule, and also the occasional appearance of a columella, which in some instances extends entirely through the center of the capsule. Because of its relation to the theory of sterilization of sporogenous tissue this plate of cells and the occasional columella should have some attention. —W. J. G. LAND.

The nucleus of bacteria.—MEYER claims[26] that the following methods will differentiate a nucleus in the bacteria. The particular form used was *Bacillus Pasteurianus*. First method: boil in water, stain 24 hours in hematoxylin, and differentiate in weak hydrochloric acid. The nuclei of young spores are sharply outlined. Second method: fix in Flemming's solution, harden in 20 per cent. alcohol, stain in Delafield's hematoxylin, and differentiate with hydrochloric acid. Third method: fix in Flemming's solution, harden in alcohol, stain in iron alum hematoxylin, and differentiate under the cover glass with ammonium ferrosulfate. Judging from the figures, this method gives the best results.

In the opinion of the reviewer, the fact that MEYER does not believe that any nucleus has as yet been demonstrated in the Cyanophyceae would not inspire confidence in his interpretation.—CHARLES J. CHAMBERLAIN.

Anatomy of Sapotaceae.—Incidentally, in seeking the origin of laticiferous tissue in Sapotaceae, Miss SMITH[27] made anatomical studies of seedlings of fourteen

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[24] BOT. GAZETTE **47**:482. 1909.

[25] DURAND, ELIAS J., The development of the sexual organs and sporogonium of *Marchantia polymorpha*. Bull. Torr. Bot. Club **35**:321–335. *pls. 21–25*. 1908.

[26] MEYER, ARTHUR, Der Zellkern der Bakterien. Flora **98**:335–340. *figs. 3*. 1908.

[27] SMITH, WINIFRED, The anatomy of some sapotaceous seedlings. Trans. Linn. Soc. London II. **7**:189–200. *pls. 25, 26*. 1909.

species distributed among eight genera. The vascular system of the primary root and hypocotyl is typically tetrarch and corresponds with two bundles from each cotyledon without change of position. In some species the root is hexarch; in others, variable and anomalous. The occurrence of the hexarch type led the author to suspect that a central cotyledonary trace, such as is found in some species of Diospyros, had aborted, but no sign of this could be found. In *Bumelia tenax* the root is usually hexarch. In the upper part of the root and the hypocotyl, four of these bundles differ from the other two in that from them alone rise the lateral rootlets, and also in that they alone are continuous with the bundles of the cotyledons.—W. J. G. LAND.

Fungus excreta.—A condition almost like that in successive cultures of the higher plants is reported for certain fungi by LUTZ.[28] He finds that in nutritive solutions in which various molds (Aspergillus, Botrytis, Cladosporium, Fusarium, Mucor, Penicillium) have been grown, there are produced substances which retard or accelerate the germination and growth of the same or other species. These products have much in common with enzymes; they are destroyed by high temperatures (80–100° C.); their action is weakened by dilution, ceasing usually at about 20-fold; they are destroyed in sunlight (20 hours), the violet rays being most efficient. Some of these substances may be stopped by a clay filter, but some pass through. The agents which accelerate growth and development are formed in lighted cultures, especially those of Fusarium and Aspergillus.—C. R. B.

Chemotropism of fungi.—As part of the larger subject, parasitism, SCHMIDT has investigated the chemotropism of an unknown species of Phyllosticta, parasitic on pear leaves.[29] He is apparently ignorant of FULTON'S work on this subject,[30] and with experimentation that is open to serious objection, comes to the conclusion that this plant is positively chemotropic. Its chemotropism, however, is not supposed to come into play at once, "but the fungus itself must first, by enzymatic, toxic, or purely mechanical means, so alter the normal structure of the epidermal cell as to set free a diffusion stream, counter to which, as a directive stimulus, the further growth of the fungus proceeds." This view he promises to support in a second paper.—C. R. B.

Culture solutions.—Any who are interested in water-cultures should consult a recent paper by BENECKE,[31] who has been testing the efficiency of VON DER CRONE'S solution, in comparison with the older ones. VON DER CRONE proposed in 1904 a solution nearly like SACHS'S, except in the addition of the iron as ferrous

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[28] LUTZ, OTTO, Ueber den Einfluss gebrauchter Nährlösungen auf die Keimung und Entwicklung einiger Schimmelpilze. Ann. Mycol. 7:91–133. 1909.

[29] SCHMIDT, E. W., Ueber den Parasitismus der Pilze. Zeit. Pflkrankh. 19:129–143. *figs.* 7. 1908.

[30] BOT. GAZETTE 41:81–108. 1906.

[31] BENECKE, W., Die von der Cronesche Nährsalzlösung. Zeits. Bot. 1:235–252. 1909.

phosphate, $Fe_3(PO_4)_2$, instead of the traces of iron which are usually added to the Sachs solution as Fe_2Cl_6. It differs from PFEFFER'S and MAYER'S essentially in the use of tricalcium phosphate, $Ca_3(PO_4)_2$, instead of potassium phosphate, thus avoiding the acidity of these solutions. BENECKE has tested VON DER CRONE'S claims, some of which he finds justified, others not. The details are not of general interest.—C. R. B.

Prochromogens.—In further development of our knowledge of plant chromogens, PALLADIN[32] has found that these substances are not present in any considerable amounts at any time, but that they are formed gradually, from what he proposes to call prochromogens, which there is some ground for thinking are glucosides. These are split up by enzymes and the chromogens are produced in small amounts, except in the spring, when larger amounts may be observed. In dead plants the enzymes give rise to large amounts of the chromogens, because the splitting is then uncoordinated, and the oxidation of these leads to the observed blackening of the tissues.—C. R. B.

Light perception.—Besides the ocelli (in the sense of HABERLANDT), SCHÜRHOFF describes[33] apparatus in six species of Peperomia which may function in the perception of light, namely: the funnelform palisade cells, by reflecting the light to the chloroplasts at their base; the upper convex wall of the palisades, by acting as a lens; and the cluster crystals, that disperse to all the chloroplasts the light focused by the lenticular upper portion of the cell. These ideas seem even more strained than the theory they are adduced to support.—C. R. B.

Wetting of leaves.—AWANO[34] furnishes the ecologists a considerable body of statistics regarding the wetability (there ought to be such a word, if there is not) of leaves. Out of 264 plants examined as to this point, he finds 164, about $\frac{2}{3}$, wetable with difficulty or not at all, while the rest are easily wetable. Leaves of most strand and sand plants are hardly wetable, while those of shade plants and ferns are easily wetable. The details, presented in extensive tables, are combined with observations on the number and distribution of stomata.—C. R. B.

Extrafloral nectaries.—SALISBURY has described[35] the extrafloral nectaries of eight species of the genus Polygonum. He ascribes the secretory action to osmotic pressure of the gland cells, independent of root pressure, and thinks that the nectar glands, which are especially striking in tropical plants, represent originally hydatodes, which have in some cases later acquired a biological significance. He

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[32] PALLADIN, W., Ueber Protochromogene der pflanzlichen Atmungschromogene. Ber. Deutsch. Bot. Gesells. **27**: 101–106. 1909.

[33] SCHÜRHOFF, P., Ozellen und Lichtkondensoren bei einigen Peperomien. Beih. Bot. Centralbl. **23**: 14–26. *pls. 3, 4.* 1908.

[34] AWANO, S., Ueber die Benetzbarkeit der Blätter. Jour. Coll. Sci. Imp. Univ. Tokyo **27**: 1–49. 1909.

[35] SALISBURY, E. J., The extrafloral nectaries of the genus Polygonum. Annals of Botany **23**: 229–242. *pl. 16. figs. 6.* 1909.

finds that there is little reason to suppose that they are of any service in protecting the flowers from ants.—C. R. B.

Centrosomes in Marchantia.[36]—After a study of spermatogenesis in *Marchantia polymorphia*, SCHAFFNER concludes that IKENO'S account is correct and that centrosomes are present, both while the nucleus is at rest and while it is undergoing mitosis. His figures are practically the same as IKENO'S. MIYAKE'S failure to find centrosomes he attributes to differences in technic.—CHARLES J. CHAMBERLAIN.

Thermotropism.—POHL[37] describes observations and experiments that he has made upon the cultivated flax, which show its great sensitiveness to radiant heat, the young shoots directing themselves toward the source. Experiments also show that heat is the dominant factor in inclination of the shoots, which is often ascribed to light.—C. R. B.

Discomycetes.—MISS BACHMAN has published[38] a descriptive catalogue of the Discomycetes within five miles of Oxford, O. Keys to genera and species are provided, and there are illustrations of ten of the sixty-odd species, a goodly number of which are now for the first time reported from southwestern Ohio.—C. R. B.

Light and respiration.—LÖWSCHIN reports[39] that when he excluded the effects of actinic warming he was unable, in the course of twenty-two experiments upon the respiration of certain fungi (Aspergillus, Penicillium, Oidium, and Cladosporium), to detect any regular acceleration of it by light.—C. R. B.

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[36] SCHAFFNER, JOHN H., The centrosomes of *Marchantia polymorphia*. Ohio Naturalist **9**:383–388. *pl. 21*. 1908.

[37] Pohl, J., Der Thermotropismus der Leinpflanze. Beih. Bot. Centralbl. **24**:111–131. *figs. 6*. 1908.

[38] BACHMAN, FREDA M., Discomycetes in the vicinity of Oxford, Ohio. Proc. Ohio State Acad. Sci. **5**:19–70. *pls. 4*. 1909.

[39] LÖWSCHIN, A., Zur Frage über den Einfluss des Lichtes auf die Atmung der niederen Pilze. Beih. Bot. Centralbl. **23**: 54–64. 1908.

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BOTANICAL GAZETTE

AUGUST 1909

EVOLUTIONARY TENDENCIES AMONG GYMNOSPERMS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 127

JOHN M. COULTER

The investigation of gymnosperms has proceeded with such vigor that some adequate picture of the group freed from its details may now be obtained. The confusion of details often obscures the important facts, and it may be of service, at this stage of our knowledge, to emphasize them. There is no better way in which to develop a clear picture of a great group than to select those facts of structure that enter into its general evolutionary history. This has nothing to do with differences among species, or even among genera, which may be left to the taxonomist; but it deals with those general tendencies to change structures which can be noted in passing from the most ancient gymnosperms to the most recent.

While the discovery of these tendencies aids in reaching conclusions in reference to the phylogenetic connections of the groups of gymnosperms, it must be remembered that the tendencies are facts and the phylogenetic conclusions are very uncertain inferences. Moreover, a general tendency expresses itself throughout a great group, and has to do with the transition from ancient to modern forms, rather than with the breaking-up of the group into several phylogenetic lines. Failure to remember this fact has been responsible for much sterile inference as to relationships, similar stages in some general tendency being assumed to mean immediate genetic connection. The organism is a plexus of structures, and must be considered in its totality when relationships are being considered. Among the general tendencies leading to the origin of seed plants, for example, that which resulted in heterospory must be regarded as of paramount importance;

and yet it is clear that heterospory arose several times, and probably many times, independently as the natural result of a general tendency among pteridophytes. To put into the same genetic group all heterosporous pteridophytes would be regarded now as a morphological absurdity; and yet there have been repeated suggestions of relationship, especially among conifers, on the basis of certain features of the female gametophyte, for example, features which represent a stage in a general change that may occur in a number of independent lines.

In passing from the ancient to the modern gymnosperms, it becomes evident that groups differ as to the rapidity with which they respond to a general tendency to change, and it, is this difference that helps to constitute groups. A modern group, for example, may associate a number of ancient features with others that are recent; or all of the ancient features may have been changed. This has been called the "lagging behind" of certain structures, but it should not imply that they are held back and will come forward later; it simply means that for some reason they have not responded to the general tendency among gymnosperms to change in a certain direction. The retention of an old structure must not be confused with the reappearance of an old structure. For example, it seems clear that the most ancient gymnosperms were large-leaved forms, from which the small-leaved conifers were derived; and yet small-leaved pteridophytes were probably more ancient than large-leaved ones. If this be true, the appearance of small leaves among conifers is the reappearance of an ancient feature, and not its retention. To prove the retention of an ancient feature demands the establishment of its phylogenetic continuity.

PHYLOGENY

Before sketching the general evolutionary tendencies among gymnosperms, it will be of service to outline what seems to be a reasonable conclusion as to the phylogenetic connections of the groups. This statement and those that follow will be all the more clear if freed from the details upon which they are based, and also from citations. Those who know the facts and the investigators do not need their recital; and those who do not know them would only be confused by their recital.

The Paleozoic groups Cycadofilicales[1] and Cordaitales represent the historical background of gymnosperms. They are of equal age, so far as the records are available, and are so connected by intergrading (or rather anastomosing) forms that their relationship seems evident. The Cycadofilicales are so fern-like in every feature except their seeds, that their derivation from some ancient fern stock (called provisionally Primofilices) is as certain as phylogenetic connections can be. The origin of the Cordaitales, therefore, presents two alternatives: either they arose independently from the same ancient fern stock, or they were differentiated from the Cycadofilicales very early. To choose between these alternatives is not very important, but the latter one seems to be the more reasonable, because the Cordaitales (as we know them) are much more removed from the ferns than are the Cycadofilicales. If this conclusion is accepted, it follows that gymnosperms began with Cycadofilicales more ancient than any yet known; that Cordaitales branched off from Cycadofilicales earlier than our present records; and that the two groups constituted the extensive gymnosperm flora of the Carboniferous.

This Paleozoic display of gymnosperms was succeeded by a Mesozoic display, in which at least four groups are recognized. From the Cycadofilicales there arose the Mesozoic Bennettitales and the Cycadales; and from the Cordaitales the Mesozoic Ginkgoales and Coniferales were derived. The Bennettitales have been traced almost to the Paleozoic, and their structure, as well as the habit of some of the earlier forms, make their connection with the Cycadofilicales appear convincing. The relation of the Bennettitales to the Cycadales is not so clear; either the two groups were differentiated from a common stock that arose from the Cycadofilicales and continued into the Mesozoic, or the Cycadales were differentiated early from the Bennettitales. The records show that the Cycadales are much younger

[1] It seems impossible at present to recognize a group Pteridosperms as distinct from Cycadofilices, and there is a growing impression that the two groups are coextensive. Under the circumstances, it would seem proper to retain for the group the older name. It seems clear, also, that it should take its place as a group of gymnosperms coordinate with Cordaitales, Cycadales, Coniferales, etc.; for it is impossible as yet to construct a definition which would exclude it from gymnosperms on the basis of characters of so high an order as those which separate gymnosperms from angiosperms. For this reason, I have appended to the older name the termination that makes it conform to the names of groups of similar rank, and shall refer to it in this paper as the CYCADOFILICALES.

than the Bennettitales, and were much more scantily represented during the Mesozoic; and therefore the latter alternative seems to be the more reasonable. In any event, the only question at issue is whether the gymnosperm stock which came from the Cycadofilicales into the Mesozoic is to be called Bennettitales or a Bennettitales-Cycadales plexus (a "common stock"); and it is altogether probable that this stock has already been assigned to the Bennettitales in the description of cycadophyte forms from the Triassic and Jurassic. In the gymnosperm flora of today, therefore, the Cycadales, although relatively a modern group, are the nearest representatives of the Paleozoic Cycadofilicales.

The Ginkgoales and Coniferales have both been traced into late and independent Paleozoic connection with the Cordaitales, and were well displayed during the Mesozoic. The Ginkgoales, while widely distributed during the Mesozoic, apparently were never a large group; and this group has continued as a single line into the present flora, and has retained certain features of the Cordaitales which the Coniferales have lost. The Coniferales, on the other hand, began that extensive differentiation during the Mesozoic which has resulted in six recognized tribes in our present flora. Among these tribes the earliest to be recognized are the Abietineae and the Araucarineae, and their very early separation is so evident as to raise the question whether they may not be independent in origin. In any event, the other tribes recognized in our present flora were of later origin; the Taxodineae and the Cupressineae, and possibly the Taxineae, arising from the Mesozoic Abietineae; and the Podocarpineae possibly arising from the Mesozoic Araucarineae.

The connections of the Gnetales are altogether obscure, and every opinion as to their origin must be regarded as very tentative. Although they have not been discovered as fossils, the great amount of differentiation they show and their widely scattered geographical distribution indicate a considerable history. Evidence seems to be accumulating that they may have been derived from the Cupressineae, or at least that they are closely related to that tribe in origin.

VASCULAR ANATOMY

The central cylinder of the Cycadofilicales, like that of ferns, was protostelic, siphonostelic, or polystelic. Whatever may be the

genetic connection of these types of cylinder, the siphonostele is the type that was carried forward in the evolution of gymnosperms. This siphonostele was made up of collateral mesarch bundles, developed secondary wood composed of tracheids, and the bundles of all its peripheral connections were concentric or at least mesarch.

Among the gymnosperms the universal tendency was to eliminate the centripetal xylem, a tendency carried forward from the ferns, until the collateral mesarch bundles of the central cylinder became collateral endarch; and more gradually the bundles of the peripheral regions became collateral mesarch and finally collateral endarch. So early was this accomplished for the central cylinder that a collateral endarch cylinder is a feature of gymnosperms in general. From what has been said as to the variable rate of change among the members of a great group, it would be expected that mesarch and even concentric bundles might be found in peripheral parts of certain species of genera whose allies had completely eliminated centripetal xylem, or might occur occasionally in any species or genus. It is an interesting fact that centripetal xylem appears to linger longest in the cotyledons; and the number of gymnosperms in which it is known to occur in this organ, regularly or occasionally, is increasing rapidly.

In the development of secondary wood, the general tendency among gymnosperms is to increase it in amount, so that a thick vascular cylinder is built up by the primary cambium. This tendency is apparent among the Cordaitales, but it reaches its most conspicuous result in the Coniferales and Ginkgoales. This changed also the general topography of the stem, both pith and cortex being much reduced in relative amount. The Bennettitales and Cycadales responded feebly if at all to this tendency, one of their features being the retention of the general stem structure of the Cycadofilicales. In these groups the primary cambium is either short-lived or functions very slowly, and in some forms secondary cambium produces cortical bundles; but the formation of secondary wood never prevents the formation of a large pith and an extensive cortex. It is in these groups also that the concentric and mesarch bundles are most common in the peripheral members, being found somewhere in all forms; while among the Coniferales the centripetal xylem has been

almost completely eliminated. In vascular anatomy therefore, the Cycadales have retained more ancient features than any other living group.

The vascular condition among Gnetales can hardly be spoken of in connection with general tendencies; but the appearance of true vessels associated with the tracheids of the secondary wood is too important to omit. In any event, these true vessels of the secondary wood suggest that in the evolution of the vascular cylinder the original tracheids of secondary wood are finally and gradually replaced by true vessels.

THE LEAF

The leaves of gymnosperms may be used to illustrate a structure that exhibits no general evolutionary tendency, but responds more or less directly to the conditions of living. The most ancient gymnosperms possessed ample, fern-like leaves, and under appropriate conditions this type of leaf persisted, as in the tropical cycads of today. The conifers, however, have developed a very different type of leaf, one that was well under way among the Cordaitales, and which reaches an extreme expression in small and rigid needles or concrescent scales. This cannot be regarded as the result of a general tendency among gymnosperms, quite unrelated to conditions of living, such as is shown by the persistent progressive changes of other structures. The leaf is too variable a structure, and too closely related in its work to external conditions to permit such an explanation of its changes.

It would be interesting to know the conditions in which needles and concrescent disks were established; but in the absence of any such knowledge, the sharply contrasted geographical distribution of Cycadales and Coniferales may suggest that the conditions of change were associated with the evolution of the land areas and of the climate of the temperate regions.

THE STROBILUS

The Cycadofilicales are the only gymnosperms without strobili. Although the sporophylls differ more or less from the fern-like foliage leaves or their branches, they are not aggregated into a strobilus distinct from the rest of the shoot. The organization of a strobilus

by the shortening of the sporophyll-bearing shoot is a conspicuous feature of gymnosperms, and it must have been derived from the condition observed among the Cycadofilicales.

The Cordaitales were the first gymnosperms to produce strobili, and this is one of their conspicuous contrasts with Cycadofilicales. The record of the structure of their strobili is meager, but it shows several tendencies in strobilus-formation. Of primary importance is the fact that the strobili are monosporangiate, and this monosporangiate character prevails throughout the Ginkgoales and Coniferales. Among the Gnetales, a group probably related to the general coniferophyte phylum, amphisporangiate strobili occur in Tumboa. If this connection be accepted, therefore, these amphisporangiate strobili have been derived from monosporangiate strobili. It is not necessary to associate in one genetic connection all of the amphisporangiate gymnosperms, for that condition doubtless appeared independently several times, just as the monosporangiate strobilus is known to have appeared at least twice in distinct phyla (Cordaitales and Cycadophytes).

Another fact in reference to the strobili of Cordaitales, which must stand for the most ancient gymnosperm strobili, is that they included both simple and compound strobili. The staminate strobilus was simple, that is, its sporophylls were borne directly upon the axis of the strobilus; and this type of staminate strobilus persisted throughout the Ginkgoales and Coniferales. Among the Gnetales the staminate strobilus is compound, the individual simple strobilus being borne on axes of the second order in the axils of sterile bracts which make up the general strobilus. There is an evident relationship between the compact compound staminate strobilus, such as occurs in Ephedra and in Tumboa, and the short foliage branch bearing axillary simple staminate strobili, as in Torreya. Even in Gnetum the compound staminate strobilus is a loose one; and among the taxads there is a tendency to compact the staminate strobiliferous branch. The conclusion is that the staminate strobilus among coniferophytes is quite persistently simple, but that in the more modern members of the phylum it tends to become compound, a condition accomplished by compacting a short strobiliferous shoot.

The ovulate strobilus of Cordaitales was compound, at least in

the very few specimens sectioned; that is, the ovules were borne on short secondary and bractlet-bearing axes that arose in the axils of the sterile and overlapping bracts that constituted the strobilus. This compound ovulate strobilus is a distinctive feature of the coniferophytes, prevailing among the Pinaceae and characterizing the Gnetales. That simple ovulate strobili may have been derived from it is quite possible. For example, in Torreya the ovulate strobili are simple and are axillary on short leafy branches, just such a branch as could have arisen through the elongation of the axis of a compound ovulate strobilus, so that the sterile bracts could be replaced by foliage leaves. It may be said that the change may have taken place in the other direction, and that the short leafy strobiliferous branch was compacted into a compound ovulate strobilus; but it must be remembered that the Cordaitales with their compound ovulate strobili are very old, and that the Taxineae with their leafy strobiliferous branches are relatively very recent. Of course it may be discovered that the Cordaitales included also forms with simple strobili on leafy shoots. This possibility is further emphasized by the fact that the ovulate strobili of the Araucarineae, and of their allies the Podocarpineae, are simple. The former tribe is a very old one, and its connection with the Cordaitales is either direct or nearly so, so that it is altogether probable that such ovulate strobili occurred in that group. The connection of the Taxineae with the Cordaitales, however, appears to be so remote, and their relation to groups with compound ovulate strobili seems to be so much more immediate, that it is more reasonable to suppose that their ovulate strobiliferous branches have arisen from compound strobili in the way described above.

Long after the Cordaitales had established their simple staminate and compound ovulate strobili, strobili appeared in the cycadophyte phylum, being found in Bennettitales and Cycadales; and even in the living Cycas the loose ovulate strobilus retains the evidence of its origin from the separated sporophylls of Cycadofilicales. The cycadophyte strobilus has always been simple, and this may be related to the more compact habit of body, with its lack of free-branching. The most remarkable feature of the early strobili of this phylum, however, is their amphisporangiate character, the two sets of sporo-

phylls holding the same relation to one another that is held by the stamens and carpels of angiosperms; and this marks the Bennettitales as a unique group among gymnosperms. The monosporangiate tendency, however, which characterizes the coniferophytes, is shown by the Cycadales among cycadophytes, and it was either established directly, or it arose from an early differentiation of the amphisporangiate strobilus of the Bennettitales. The evidence of history favors the latter view, but the probabilities of the situation favor the former. In any event, both monosporangiate and amphisporangiate strobili were established among cycadophytes.

THE STAMEN

Among gymnosperms the stamen may be regarded as a very conservative structure, retaining throughout most of the phyla its fern-like characteristics. Its form perhaps became almost as much differentiated among the Cycadofilicales as it ever became among gymnosperms. In this primitive group, in addition to microsporophylls resembling fern-like leaves with abaxial synangia, there appeared others that have been spoken of as the Crossotheca ("epaulet") and the Calymmatotheca ("cupule") types, and in all probability still others will be discovered. All of these types were continued among the coniferophytes, with varying details of minor importance. Among some of the Gnetales, either the sporophyll has become very much reduced, or it has become suppressed, so that the microsporangia are cauline; but even in Tumboa the old terminal synangium is evident.

Among the cycadophytes, on the other hand, only what may be regarded as the most ancient type of microsporophyll has been retained, that is, the fern-type with abaxial sporangia (often synangia). Among the Bennettitales, there is so little departure from the old type that their microsporophylls resemble pinnate fern leaves with abaxial synangia; and even among the Cycadales the more or less leaf-like microsporophylls show the same character. If there is any tendency in the stamens of this phylum worth noting, it is the tendency shown among the cycads to reduce the sterile apex of the sporophyll to a more compact peltate expansion.

The microsporangium of gymnosperms is a very consistent struc-

ture, originating from the hypodermal layer of cells, and developing a wall of several layers, the innermost one of which is usually differentiated as the tapetum. The only general tendencies to be observed are the gradual replacement of synangia by separate sporangia, and the more rapid elimination of all evidences of an annulus (in the general sense). It is noteworthy that in both these particulars the cycadophytes, with their much more recent connection with the Cycadofilicales, are far behind the coniferophytes.

THE OVULE

The origin of the ovule of gymnosperms remains in obscurity. While the stamen and its sporangia repeat the corresponding structures of ferns, the ovules of Cycadofilicales and of Cordaitales are so well organized, even in the modern sense, that their connection with the sporangia or synangia of ferns is entirely a matter of inference. This means a tremendous gap between the somewhat hypothetical Primofilices, on the one hand, and the Cycadofilicales and Cordaitales, as we know them, on the other hand, a gap which there seems to be small probability of filling up with intermediate forms. In this presentation the only thing possible is to take the ovules of the investigated Paleozoic forms as representing the oldest known ovules, and to note the general changes that have occurred since.

To select the most primitive type of ovule from among the Paleozoic forms that have been investigated is impossible, unless it is assumed that those ovules which are most unlike the modern ones represent the most primitive type. This may or may not be true, but it is the only available criterion; and by using it, we obtain the following result. The oldest ovule had a single integument entirely free from the nucellus; in testa-formation this integument differentiated into three layers, the outer fleshy, the stony, and the inner fleshy; the ovule was supplied with two sets of vascular strands, the outer set traversing the outer fleshy layer, and the inner set traversing the peripheral region of the nucellus; and the beaked tip of the nucellus broke down more or less completely within the firm and resistant epidermis to form a pollen chamber. If these are really the features of the most primitive known ovules, the changes become very apparent, and they represent general tendencies, for they appear in every phylum.

In the first place, the integument and nucellus, instead of remaining separate, develop separately only in the region of the nucellar beak. So early was this change that it probably represents the condition of the majority of the Paleozoic ovules, a condition which has persisted ever since. The method of development is very evident, the integument appearing first as a distinct annular growth about the base of the young nucellus, but later its basal meristematic zone becoming indistinguishable from that of the nucellus. In all probability the change was brought about by the earlier appearance of the integument, and the result has been more or less variability in the amount of freedom from the nucellus.

The three-layered testa persists remarkably throughout gymnosperms, varying chiefly in the amount of development of the outer fleshy layer. The stony layer is always strongly developed, and at the maturity of the seed the inner fleshy layer always forms for it a papery lining. A strong development of the outer fleshy layer, resulting in fleshy seeds, continues throughout the cycadophyte phylum and the Ginkgoales and is a feature of many of the Taxaceae. Among the Pinaceae the outer fleshy layer is present in the young integument, but does not develop, so that the stony layer is the conspicuous superficial feature of the seed. The development of the outer fleshy layer among the Cycadales and Ginkgoales is phylogenetically continuous from the Cycadofilicales and Cordaitales; but among the Taxaceae there is probably no such continuity, but a reappearance of the activity of this layer in certain genera. Among the Gnetales, the single integument of the other gymnosperms is replaced by two integuments, the inner fleshy layer having become differentiated as a delicate inner integument, which appears earlier than the heavier outer integument, which gives rise to the outer fleshy and stony layers. In Gnetum the outer fleshy layer develops the pulpy investment characteristic of the primitive seeds; but in Ephedra this layer behaves as among the Pinaceae. If any general tendencies can be inferred from these facts in reference to the integument and testa they are seen in the abortion of the outer fleshy layer in the largest group of living gymnosperms, and in the final differentiation of the three-layered integument into two integuments.

The vascular supply of the ovule exhibits a very evident progressive

change. When the integument and nucellus become free only in the region of the nucellar beak, the inner set of vascular strands is shifted from the peripheral region of the nucellus to the inner fleshy layer, and this situation persists among the cycadophytes. Curiously enough, it reappears in Gnetum, but in that case it is associated with the presence of the two integuments. Among Ginkgoales the outer set of strands is suppressed; among Taxaceae the inner set is suppressed; and among Pinaceae both sets have disappeared. The general tendency, therefore, is to eliminate the vascular strands from the ovule; but it is puzzling to find both sets absent from the older Abietineae, and one set still present among the younger Taxineae.

The presence of a pollen chamber is one of the most conspicuous features of the primitive ovule, and its association with fertilization by ciliated sperms is so evident that it is natural that the two disappeared simultaneously with the establishment of Coniferales, but the abruptness of the disappearance is evidently more apparent than real. The presence of an extraordinarily deep pollen chamber in Ephedra can hardly be regarded as a contradiction to this general statement, for in that case it is evidently of secondary origin, associated with a remarkably massive archegonium neck.

THE FEMALE GAMETOPHYTE

The female gametophyte of gymnosperms exhibits a progressive series of changes which is significant because it leads toward the angiosperm condition. At this point the very important historical record fails, and the entire testimony must be obtained from living forms, which do not represent a series, but the ends of many series. For this reason, and also because such progress is always very unequal in different forms, various stages of advancement may be expected to be found in forms grouped in a single alliance. The series, therefore, is not so much one which conforms to recognized groups, as a series of stages each of which may be exhibited by members of various groups.

The general development of the gametophyte is quite uniform; and since the same sequence of events occurs among the heterosporous pteridophytes, it may be inferred that a knowledge of the ancient

gymnosperms would not materially change this situation. The general sequence referred to in the development of the gametophyte is as follows: free nuclear division, usually accompanied by vacuolation which results at some stage in parietal placing; the formation of walls, resulting in a parietal tissue; the centripetal growth of this tissue until it reaches the center of the embryo sac; and the final growth of the gametophyte until it reaches its mature size. In certain cases there may be no parietal placing, the free nuclei remaining distributed throughout the embryo sac; and therefore there is no centripetal growth, but general wall-formation throughout the sac. The details of the formation of permanent endosperm tissue from the primary walled cells are variable and perhaps very important from the evolutionary point of view, but the range of forms from which these details have been obtained is far too small to make them of present service in this connection.

The tendency which runs through gymnosperms as a whole, and which reaches its extreme expression among angiosperms, is to mature the eggs earlier and earlier in the ontogeny of the gametophyte. In the most primitive condition of the gametophyte, the eggs do not appear until the endosperm is nearly full grown; and other gametophytes can be selected and arranged in a series showing the gradual slipping-back of the egg in the ontogeny of the gametophyte, until in such a form as Torreya the archegonium initial is differentiated as soon as wall-formation has taken place. A conspicuous illustration of the inequality of response to such a general tendency among related forms is furnished by Torreya and Cephalotaxus, the archegonia not appearing in the latter genus until the gametophyte is well grown. The next stage is illustrated by the situation in Tumboa, in which eggs are matured before wall-formation is complete, resulting in the elimination of archegonia. The extreme stage in this progressive series of changes among gymnosperms is illustrated by Gnetum, in which eggs are matured at the stage of free nuclear division, the most embryonic stage of the female gametophyte.

So far as the living forms are concerned, the Cycadales and Ginkgoales show little, if any, response to this tendency; and therefore possess the most primitive type of female gametophyte among living gymnosperms. Among the Coniferales, on the other hand,

all stages are found, to the one just preceding the elimination of archegonia; and this stage is attained by Tumboa and Gnetum.

The general tendency of the archegonia among gymnosperms is to eliminate the ventral canal cell. The gymnosperms are distinguished from the pteridophytes by the complete elimination of neck canal cells, and this tendency to suppress all of the axial row except the egg continues among gymnosperms. Among the living forms, a walled ventral canal cell is retained only among the Abietineae, so far as known; but it seems to be a safe inference that it was present among the ancient gymnosperms. In the other living groups the wall has disappeared, and the ventral canal cell is represented by a free nucleus. In certain forms even this nucleus may have disappeared; and of course there is no trace of it when the archegonia are eliminated.

The distribution of archegonia may be considered in this connection, although the tendencies do not appear general. It is becoming evident that the position of archegonia is related to the position of the pollen tube, which often reaches the embryo sac before the archegonium initials are selected. In cases where the pollen tube assumes a lateral position in reference to the gametophyte (as in Sequoia and Widdringtonia), it has been demonstrated that the latter responds by the selection of numerous deep-seated and laterally placed archegonium initials. It may be inferred, therefore, that the usual micropylar position of archegonia is due to the usual micropylar position of the tip of the pollen tube. It may be that numerous scattered and rather indefinitely placed archegonia were a feature of ancient gymnosperms, but there is no evidence for it; on the contrary, the few sections of Paleozoic ovules that reveal archegonia, and also the archegonia of heterosporous pteridophytes, suggest the opposite conclusion. In any event, they tend to become definite in number and are then organized in two ways: either as individual archegonia, each with its own jacket and chamber; or as an archegonial complex, with a common jacket and chamber. The latter may seem to be a specialized condition, exhibited chiefly by Cupressineae, but it also seems to be the natural condition from which to derive the free eggs of Tumboa and Gnetum when archegonia are eliminated from ontogeny.

THE MALE GAMETOPHYTE

It is perhaps impossible as yet to determine the character of the male gametophyte of the Paleozoic gymnosperms. The evidence is accumulating that it comprised many more cells than do the gametophytes of most living gymnosperms; but it is not demonstrable whether these supernumerary cells were vegetative or spermatogenous. There are instances of supernumerary cells of both kinds among living gymnosperms, so that they furnish no clue; and the same is true of heterosporous pteridophytes. The balance of probability, however, is in favor of the view that they were in the main spermatogenous.

In any event, starting with the known condition among heterosporous pteridophytes, the tendency among gymnosperms has been to reduce and finally to eliminate the vegetative (prothallial) tissue; and to reduce the sperm mother cells to two.

In certain groups (as Abietineae) the prothallial cells are two in number; in others (as cycads) there is one prothallial cell; and in still others (as Taxodineae, Cupressineae, and Taxineae) prothallial tissue has been eliminated. Such prothallial cells as do appear are sometimes persistent and sometimes ephemeral; so that the evidence of a disappearing tissue is complete, and it actually has disappeared in what are recognized as the most modern groups. The situation common to Podocarpineae and Araucarineae is usually cited as an illustration of a more extensive and therefore a more ancient prothallial tissue, which connects directly with the "multicellular" pollen grains of Cordaitales. This may be true, but all of the extra cells are derived from two primary ones, which hold a definite place in the ontogeny of the gametophyte; and therefore may represent a secondary tissue that holds no phylogenetic relation to the more extensive prothallial tissues of older forms. In any event, it is ephemeral, breaking down and liberating its nuclei.

The number of sperm mother cells is so rigidly two, that this reduction may be said to have been accomplished by all living gymnosperms, whatever may be the fact in reference to the Paleozoic gymnosperms. It is interesting to note that the very few instances of a greater number of sperm mother cells occur in one group characterized by its retention of ancient features (Cycadales), and in another

group characterized by its very modern features (Cupressineae). While phylogenetic continuity of multiple sperms might be claimed for Cycadales, no such claim could be maintained for Cupressineae.

The greatest epoch in the history of the male gametophyte of gymnosperms, however, was the abandonment of ciliated sperms, and this occurred in connection with the establishment of Coniferales. It is not generally appreciated that five of the seven recognized primary groups of gymnosperms possess ciliated sperms, and that the modern type or completely terrestrial type of sperm was introduced by the conifers. This also affected the pollen chamber, the pollen tube, and the cell generations in spermatogenesis. The pollen chamber disappeared; the pollen tube ceased to be exclusively a branching haustorial organ and became a sperm-carrier; while the last cell-generation in spermatogenesis was omitted. It is this condition of spermatogenesis that is carried forward by angiosperms to a still greater stage of reduction. Just what cells are eliminated and what cells remain is a question of small importance. The significant fact is that spermatogenesis is shortened, and the ultimate cells, although non-ciliated, are physiologically sperms.

THE EMBRYO

The absence of embryos from the seeds of Paleozoic gymnosperms indicates that some great change connected with embryo-formation was introduced by the Mesozoic gymnosperms. It would be of extreme interest to know the ancient condition, but we know only what it has become. After this change, whatever it may have been, the proembryo has become the structure showing steady and progressive change.

The first stage in the development of the proembryo is free nuclear division, followed by wall-formation; and in the most primitive condition the free nuclei are so numerous that wall-formation results in a tissue which fills the egg. The tendency is to reduce the number of free nuclear divisions, resulting in a reduction of the amount of proembryonic tissue, so that more and more of the general cytoplasm of the egg is left free from tissue. The proembryo of Ginkgo has retained a very primitive character; and illustrations of early stages in the reduction of the proembryo may be observed among the cycads.

In Zamia, for example, which illustrates the extreme amount of reduction among cycads, the proembryo is a relatively small amount of tissue at the base of the egg. Among the Coniferales this reduction has been carried forward to a much greater extent, 16 free nuclei appearing sometimes, 8 free nuclei being the prevailing number, and 4 free nuclei being occasionally attained. This results in a proembryo consisting of a small and definite number of cells, with distinctly distributed functions. Now and then among gymnosperms (as in Sequoia), there is no free nuclear division, but these are interesting rather than significant exceptions. Even among the Gnetales it is found that a certain amount of free nuclear division precedes wall-formation. That this reduction of the free nuclei before wall-formation is significant, is shown by the fact that among angiosperms free nuclei have disappeared, and wall-formation accompanies the first division of the egg-nucleus.

THE UNIVERSITY OF CHICAGO

ON SIMILARITY IN THE BEHAVIOR OF SODIUM AND POTASSIUM

W. J. V. Osterhout

(WITH FOUR FIGURES)

It is commonly mentioned by textbooks, as worthy of remark, that sodium and potassium agree closely in chemical behavior, but differ fundamentally in their effects upon plants.

This general statement is founded on the study of the nutritive functions of sodium and potassium. There is no a-priori reason for supposing it to be true in the field of toxic or of protective action. As this is a point of general interest I have made some experiments with reference to it.

Two extensive series of experiments, one on sodium, the other on potassium, were carried on simultaneously. They were found to show a remarkable degree of agreement in the action of these two substances.

The experiments relating to sodium have already been described,[1] while those on potassium have been withheld from publication, pending thecompletion of further observations on the mutually antagonistic action of sodium and potassium.

Most of the experiments were made with a variety of wheat known as Early Genesee. The technique has been fully described in a previous paper.[2]

TOXIC ACTION

In the earliest studies which I made on balanced solutions, I was struck with the fact that Na and K agree closely in their toxic effect on plants.

These results I have found to hold in an extensive series of experiments, including algae, liverworts, Equisetum, and some thirteen genera of flowering plants. While there are doubtless some exceptions, the general rule seems to be that Na and K are closely similar in their toxic action.

[1] Jahrb. Wiss. Bot. **46**:121. 1908.

[2] Bot. Gazette **44**:266. 1907.

The most careful quantitative studies which we possess on this point are those of Miss MAGOWAN.[3] These studies show that the toxicity curves for K and Na are practically identical, while the corresponding curve for Mg shows a much higher, and that for Ca a much lower degree of toxicity.

ANTAGONISTIC ACTION[4]

The antagonistic action of monovalent kations on each other has especial interest in view of the experiments of LOEB[5] on Fundulus, which offer a certain analogy with those of LINDER and PICTON.[6] In these experiments monovalent kations antagonized bivalent but did not antagonize other monovalent kations.

The curve of antagonism between NaCl and KCl shows two maxima. The location of these maxima, however, is not constant, but varies somewhat in different series of experiments. Table I and *fig. 1* show the average result of four series. The antagonism

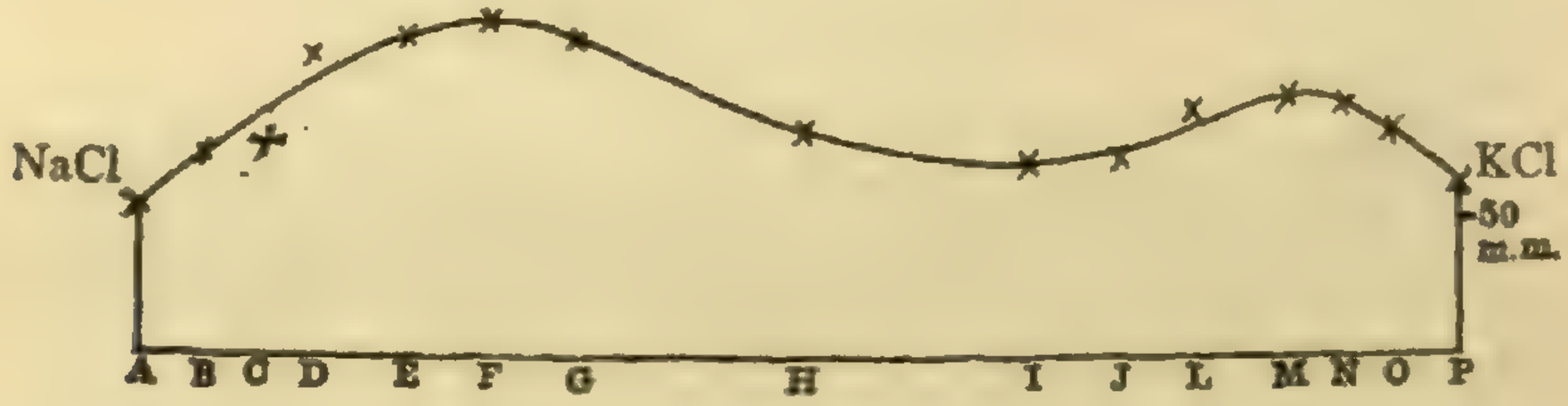

FIG. 1.—Antagonism curve, NaCl vs. KCl. The ordinates represent millimeters of growth of the roots of wheat. The ordinate at A represents the growth in pure NaCl, that at P the growth in pure KCl. The other ordinates represent growth in mixtures of NaCl and KCl, the proportions of which are found opposite the corresponding letters in table I: thus the ordinate at H represents growth in a mixture of 100cc NaCl + 100cc KCl.

is comparatively slight. I have also noticed antagonism between Na and K in liverworts.

Table II and *fig. 2* show that both Na and K antagonize NH_4, and that their effects are very similar.

[3] *Ibid.*, **45**:45. 1908.

[4] Cf. facts and literature given by KEARNEY and CAMERON, Rept. No. 71, U. S. Dept. Agr. 1902; and by BENECKE, Ber. Deutsch. Bot. Gesells. **25**:322. 1907.

[5] American Journal of Physiology **3**:327. 1900.

[6] Cf. HOBER UND GORDON, Hofmeister's Beitr. Chem. Physiol. und Pathol. **5**:432. 1904.

TABLE I

WHEAT (GROWTH DURING 30 DAYS). ALL QUANTITIES GIVEN ARE CUBIC CENTIMETERS OF 0.12 *m* SOLUTIONS

Culture solution	Corresponding point on curve (*fig. 1*)	Aggregate length of roots per plant in mm
NaCl.....	A	55
100 NaCl, 5 KCl	B	75
100 NaCl, 10 KCl	C	80
100 NaCl, 15 KCl	D	115
100 NaCl, 25 KCl	E	120
100 NaCl, 35 KCl	F	130
100 NaCl, 50 KCl	G	121
100 NaCl, 100 KCl	H	85
50 NaCl, 100 KCl	I	75
35 NaCl, 100 KCl	J	80
25 NaCl, 100 KCl	L	95
15 NaCl, 100 KCl	M	100
10 NaCl, 100 KCl	N	95
5 NaCl, 100 KCl	O	85
KCl.....	P	65
Distilled water, 725mm		

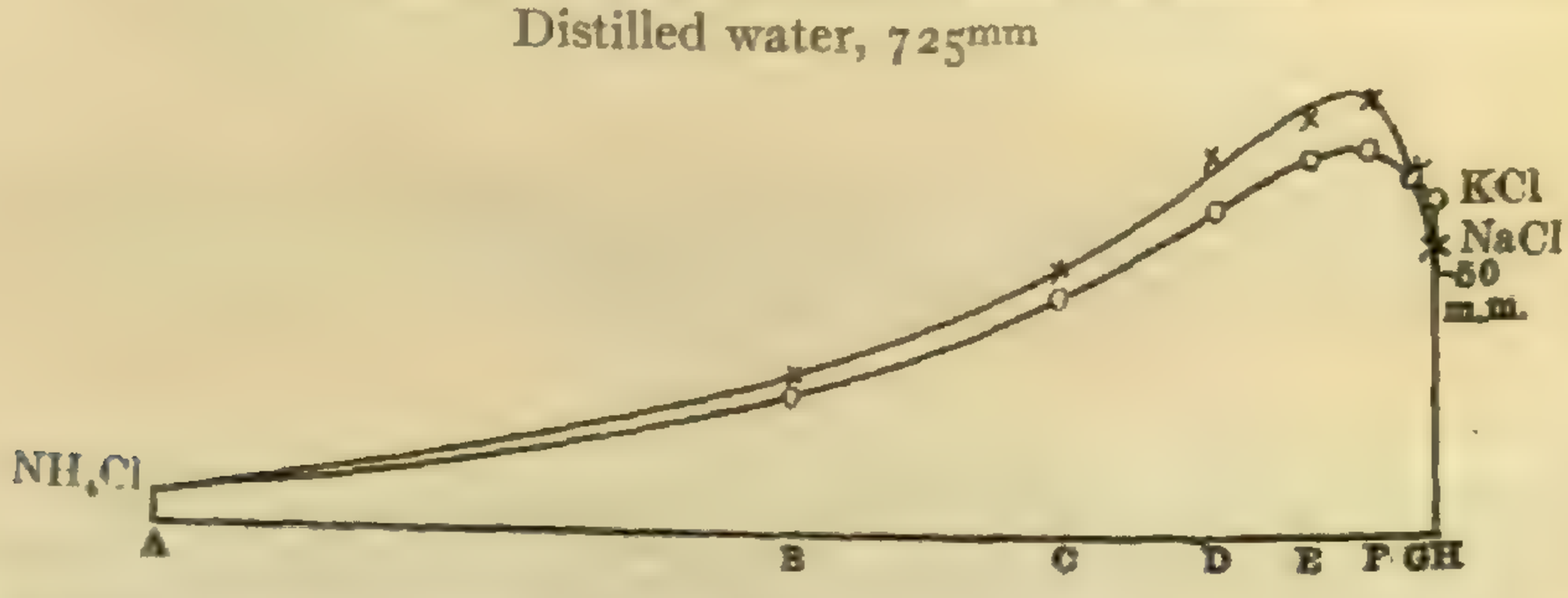

FIG. 2.—Antagonism curve, NH_4Cl vs. NaCl (upper curve -x-x-x-) and NH_4Cl vs. KCl (lower curve -o-o-o-). Each ordinate represents the amount of growth of wheat roots in a solution whose composition is given opposite the corresponding letter in table II.

TABLE II

WHEAT (GROWTH DURING 30 DAYS). ALL QUANTITIES GIVEN ARE CUBIC CENTIMETERS OF 0.12 *m* SOLUTIONS

Culture solution	Aggregate length of roots per plant in mm	Corresponding point on curve (*fig. 2*)	Culture solution	Aggregate length of roots per plant in mm
NHC_4l.....	6.2	A	NH_4Cl.....	6.2
100 NH_4Cl } ..... 100 NaCl }	31	B	100 NH_4Cl } ..... 100 KCl }	27.5
40 NH_4Cl } ..... 100 NaCl }	52	C	40 NH_4Cl } ..... 100 KCl }	46
20 NH_4Cl } ..... 100 NaCl }	74	D	20 NH_4Cl } ..... 100 KCl }	62
10 NH_4Cl } ..... 100 NaCl }	80	E	10 NH_4Cl } ..... 100 KCl }	72.5
5 NH_4Cl } ..... 100 NaCl }	85	F	5 NH_4Cl } ..... 100 KCl }	75
1 NH_4Cl } ..... 100 NaCl }	67	G	1 NH_4Cl } ..... 100 KCl }	68
NaCl.....	55	H	KCl.....	66

Distilled water, 725 mm

Experiments with magnesium show that it is antagonized in about the same degree by both Na and K (table III and *fig. 3*).

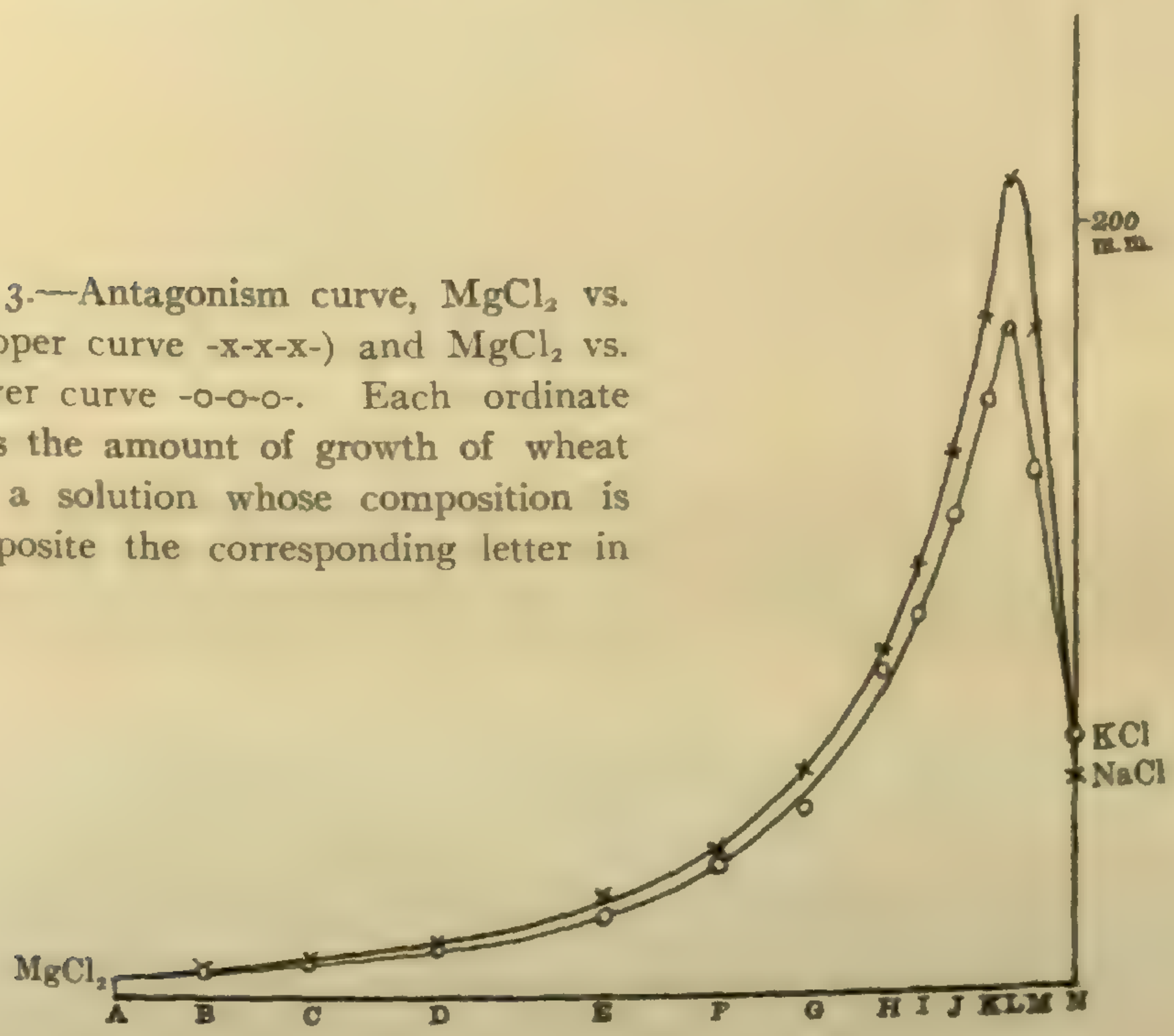

FIG. 3.—Antagonism curve, $MgCl_2$ vs. NaCl (upper curve -x-x-x-) and $MgCl_2$ vs. KCl (lower curve -o-o-o-. Each ordinate represents the amount of growth of wheat roots in a solution whose composition is given opposite the corresponding letter in table III.

TABLE III

WHEAT (GROWTH DURING 30 DAYS). ALL QUANTITIES GIVEN ARE CUBIC CENTIMETERS OF 0.12 *m* SOLUTIONS

Culture solution	Aggregate length of roots per plant in mm	Corresponding point on curve (*fig. 3*)	Culture solution	Aggregate length of roots per plant in mm
$MgCl_2$	5	A	$MgCl_2$	5
100 $MgCl_2$ + 10 NaCl	7·5	B	100 $MgCl_2$ + 10 KCl	7·5
100 $MgCl_2$ + 25 NaCl	10	C	100 $MgCl_2$ + 25 KCl	8.7
100 $MgCl_2$ + 50 NaCl	13·7	D	100 $MgCl_2$ + 50 KCl	12.5
100 $MgCl_2$ + 100 NaCl	25	E	100 $MgCl_2$ + 100 KCl	21
60 $MgCl_2$ + 100 NaCl	37·5	F	60 $MgCl_2$ + 100 KCl	34
40 $MgCl_2$ + 100 NaCl	50.7	G	40 $MgCl_2$ + 100 KCl	48.7
25 $MgCl_2$ + 100 NaCl	90	H	25 $MgCl_2$ + 100 KCl	84
20 $MgCl_2$ + 100 NaCl	112	I	20 $MgCl_2$ + 100 KCl	99
15 $MgCl_2$ + 100 NaCl	140	J	15 $MgCl_2$ + 100 KCl	125
10 $MgCl_2$ + 100 NaCl	176	K	10 $MgCl_2$ + 100 KCl	152
7·5 $MgCl_2$ + 100 NaCl	210	L	7·5 $MgCl_2$ + 100 KCl	171
5 $MgCl_2$ + 100 NaCl	172	M	5 $MgCl_2$ + 100 KCl	135
NaCl.....	55	N	NaCl.....	66

Distilled water, 725 mm

In algae I have found that $MgCl_2$ is much more strikingly antagonized by KCl than by NaCl.

The experiments with calcium show a more marked antagonism than any of the other cases. We find that Ca is antagonized to a slightly greater degree by K than by Na (table IV and *fig. 4*).

Similar results were obtained with algae, liverworts, Equisetum, and some fifteen genera of flowering plants.

From the facts here set forth it is clear that in their toxic and

protective effects sodium and potassium show great similarity. As this does not seem to be the case in the field of nutritive effects, we

FIG. 4.—Antagonism curve, $CaCl_2$ vs. KCl (upper curve -x-x-x-) and $CaCl_2$ vs. NaCl (lower curve o-o-o). Each ordinate represents the amount of growth of wheat roots in a solution whose composition is given opposite the corresponding letter in table IV

seem to have in this case a means of distinguishing clearly between nutritive and protective action.

TABLE IV

WHEAT (GROWTH DURING 30 DAYS). ALL QUANTITIES GIVEN ARE CUBIC CENTIMETERS OF 0.12 *m* SOLUTIONS

Culture solution	Aggregate length of roots per plant in mm	Corresponding point on curve (*fig. 4*)	Culture solution	Aggregate length of roots per plant in mm
$CaCl_2$	85	A	$CaCl_2$	85
100 $CaCl_2$ + 10 NaCl	100	B	100 $CaCl_2$ + 10 KCl	105
100 $CaCl_2$ + 25 NaCl	117	C	100 $CaCl_2$ + 25 KCl	125
100 $CaCl_2$ + 50 NaCl	150	D	100 $CaCl_2$ + 50 KCl	174
100 $CaCl_2$ + 100 NaCl	198	E	100 $CaCl_2$ + 100 KCl	230
50 $CaCl_2$ + 100 NaCl	262	F	50 $CaCl_2$ + 100 KCl	337
25 $CaCl_2$ + 100 NaCl	342	G	25 $CaCl_2$ + 100 KCl	420
15 $CaCl_2$ + 100 NaCl	390	H	15 $CaCl_2$ + 100 KCl	464
10 $CaCl_2$ + 100 NaCl	416	I	10 $CaCl_2$ + 100 KCl	492
5 $CaCl_2$ + 100 NaCl	440	J	5 $CaCl_2$ + 100 KCl	507
1 $CaCl_2$ + 100 NaCl	300	K	1 $CaCl_2$ + 100 KCl	346
NaCl.....	55	L	KCl.....	66

Distilled water, 725 mm

SUMMARY

The accepted idea that sodium and potassium have entirely different effects upon plants is not valid in the field of toxic and protective action. Here their behavior shows the close similarity which their near chemical relationship would lead us to expect.

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TOXIC AND ANTAGONISTIC EFFECTS OF SALTS AS RELATED TO AMMONIFICATION BY *BACILLUS SUBTILIS*

CHAS. B. LIPMAN

(WITH FIVE FIGURES)

In the science of bacteriology, especially in that of soil bacteriology, our work has thus far only taught us enough to give added zest to investigation and the most important and interesting results are still forthcoming. RAMANN (**23**) well expresses it when he says, "In spite of numerous and important investigations we are still but in the first stages of our researches on bacteria and only at the very starting-point in our knowledge of soil bacteria." In his haste to classify bacteria, to show their direct relation to many diseases, to apply his knowledge of them to the practical side of the dairy industry, and finally, to measure the net results of the activities of bacteria in the soil, the bacteriologist has left almost untouched one of the most important phases of the science of bacteriology, namely, the physiology of bacteria, which is of great scientific interest and practical importance. Especially is this true of the physiology of soil bacteria, which remains as yet a closed book, and since the writer is devoting his time to researches on soil bacteria in particular, it was thought best at first to experiment with some of these organisms. It may also be added that in California, with its thousands of acres of waste alkali land, and in similar regions elsewhere this study will undoubtedly prove to be of the greatest practical significance, especially when we have learned to coordinate the results obtained in similar investigations on the higher plants with those derived from researches on soil bacteria.

Since ammonification is the first great step in the transformation and simplification of the organic soil nitrogen, it was thought best to study the effect of various salt solutions on pure cultures of ammonifiers first. The work was carried out along the same general lines as to the preparation of solutions as the experiments of LOEB on the effects of salt solutions on various forms of animal life, and the subse-

quent experiments of OSTERHOUT on the higher plants. It was indeed the insight of the latter investigator which led him to conclude some time ago that a proper understanding of physiologically balanced solutions in relation to plants and to soil bacteria would render the control and successful cultivation of alkali lands a much simpler matter than it has been, and this will undoubtedly prove true in the near future.

In the selection of an ammonifier which could be used uniformly throughout all the experiments, the writer was guided by the work of MARCHAL (**15**), to whom indeed we owe what little knowledge we have of the physiology of ammonifiers. Among the best ammonifiers found by that investigator were *B. mycoides*, which changed 46 per cent. of nitrogen into ammonia in a given time, *Proteus vulgaris* (36 per cent.), *Sarcina lutea* (27 per cent.), and *B. subtilis* (19 per cent). Since the last form is easily isolated and cultivated and is a strong ammonifier, it was decided to use it in the following experiments. It is more than probable that the same relative results will be obtained with any ammonifier; this, however, will be tested in other experiments now contemplated by the writer. The pure culture of *B. subtilis* employed for inoculation through all the series of experiments was obtained from soil from Auburn in the foothill fruit region of California.

The salts tested were the chlorids of sodium, potassium, calcium, and magnesium. Only chemically pure salts were used, after submitting them to a flame test. Molecular or bimolecular stock solutions in distilled water were made, from which the requisite amounts were taken for the various concentrations. Witte's peptone was the nitrogenous substance used for the ammonification, of which 1 per cent. solutions were employed in the tests for the single salts and 0.75 per cent. in the binary solutions. The method of inoculation employed was as follows: Inoculation is made from peptone agar slope of *B. subtilis* into a sterile 100^{cc} portion of 1 per cent. peptone in a 250^{cc} Erlenmeyer flask. This is incubated for forty-eight hours at 28° C., at the end of which time the membrane that forms on the surface of the culture is precipitated by slight shaking, and then by tilting the flask to one side and carefully setting it down again, the liquid covering part of the bottom of the flask remains free from membranous

material and is homogeneous in character. Of this homogeneous liquid 1^{cc} was drawn off with a sterile pipette for inoculation into each flask to be tested, the greatest caution being used to prevent any particle of membrane from entering the pipette as the liquid was drawn up. This was the most satisfactory method of inoculation of several tested and yields concordant results in the duplicate series. All the solutions employed were made practically neutral. The incubation was carried out in a thermostat at a temperature which varied between 28° and 29° C. The incubation period was two days in the case of the single salt solutions, and two and one-half days for the binary solutions. The amount of ammonia formed, which was used as a criterion for establishing the efficiency of *B. subtilis* in the various solutions, was determined as follows: At the end of the incubation period the culture solutions were transferred to flat-bottomed Jena distillation flasks, diluted to 300–350^{cc}, an excess of magnesium oxid added, and distilled. The amount of ammonia in the distillate was titrated against standard acid, cochineal being used as the indicator. Sterile blanks were run on all determinations, each of which was made in duplicate, and the tables given below represent averages of at least three sets of such duplicates and in some instances of five and six sets of duplicates.

Experiments with single salts

In determining the salts to be tested the writer was guided by the alkali and alkaline earth constituents of soils. The sodium, potassium, calcium, and magnesium salts are important factors in plant nutrition and are always present in soils; in some cases, indeed, one or more of them may be present in such excess as to inhibit plant growth materially and in some instances completely. Such soils we find in California and other states under the common appellation of "alkali lands." It was decided, therefore, to test the salts of the alkalies and alkaline earths above mentioned, to determine the degree of toxicity of each for the bacteria experimented upon. Since from similar work on animals and plants, the anion of salts was found to have comparatively little effect, a chlorid of each metal was employed for the sake of uniformity.

SERIES I. SODIUM CHLORID

There were prepared sixteen Erlenmeyer flasks (125^{cc} capacity), each containing 50^{cc} of solution, made up as follows: The first flask contained 50^{cc} of 1 per cent. peptone solution in distilled water; the second contained a 0.1 *m* solution of NaCl and 1 per cent. peptone; the third flask contained 0.2 *m* NaCl and 1 per cent. peptone; and so on, the concentration of NaCl increasing by 0.1 *m* in each succeeding flask up to the sixteenth, which contained 1.5 *m* NaCl and 1 per cent. peptone. All the flasks were plugged with cotton, sterilized in the autoclave at a pressure of 1.25 atmospheres, and when cool were inoculated from a culture of *B. subtilis* in the manner above described. After two days' incubation at 28° to 29° C., the ammonia formed in the peptone solutions was distilled off and determined as above explained. Table I shows the results.

TABLE I

Numbers represent tenths *m* NaCl solution	Milligrams of N formed as NH_3
0	5.60
1	6.79
2	4.13
3	2.80
4	2.59
5	2.45
6	2.38
7	2.10
8	1.89
9	1.54
10	1.33
11	1.05
12	0.91
13	0.28
14	0.28
15	0.28

Plotting a curve by laying off the concentrations as abscissae and the milligrams of N (as NH_3) as ordinates (*fig. 1*), we note the following: NaCl up to a concentration of 0.1 *m* stimulates *B. subtilis* as an ammonifier, but beyond that concentration it becomes gradually more and more toxic, and at 1.3 *m* we find scarcely any ammonification. It is evident, also, that NaCl is not nearly as toxic for *B. subtilis* as it has been found by LOEB (**8,** p. 412)[1] and OSTWALD (**21**) to be for animals, and as OSTERHOUT (**17**) and MAGOWAN (**14**) have found it to be for plants. LOEB found, for instance, that the formation of embryos in the eggs of Fundulus was rendered impossible in a 0.625 *m* solution of NaCl, whereas in a 0.7 *m* solution of the same salt *B. subtilis* makes a fairly good growth and forms a considerable amount of ammonia. As an extreme example of the toxicity of NaCl, OSTERHOUT (**18**) found that zoospores of *Vaucheria sessilis* placed in a 0.0937 *m* solution of NaCl usually died within a few min-

[1] See also 5 and literature there cited.

utes, and further, that even at a dilution of 0.0001 *m* NaCl proved poisonous to the young plants. The results above given serve, however, to confirm again the general principle, formulated by LOEB, of the toxicity of NaCl alone for living organisms.

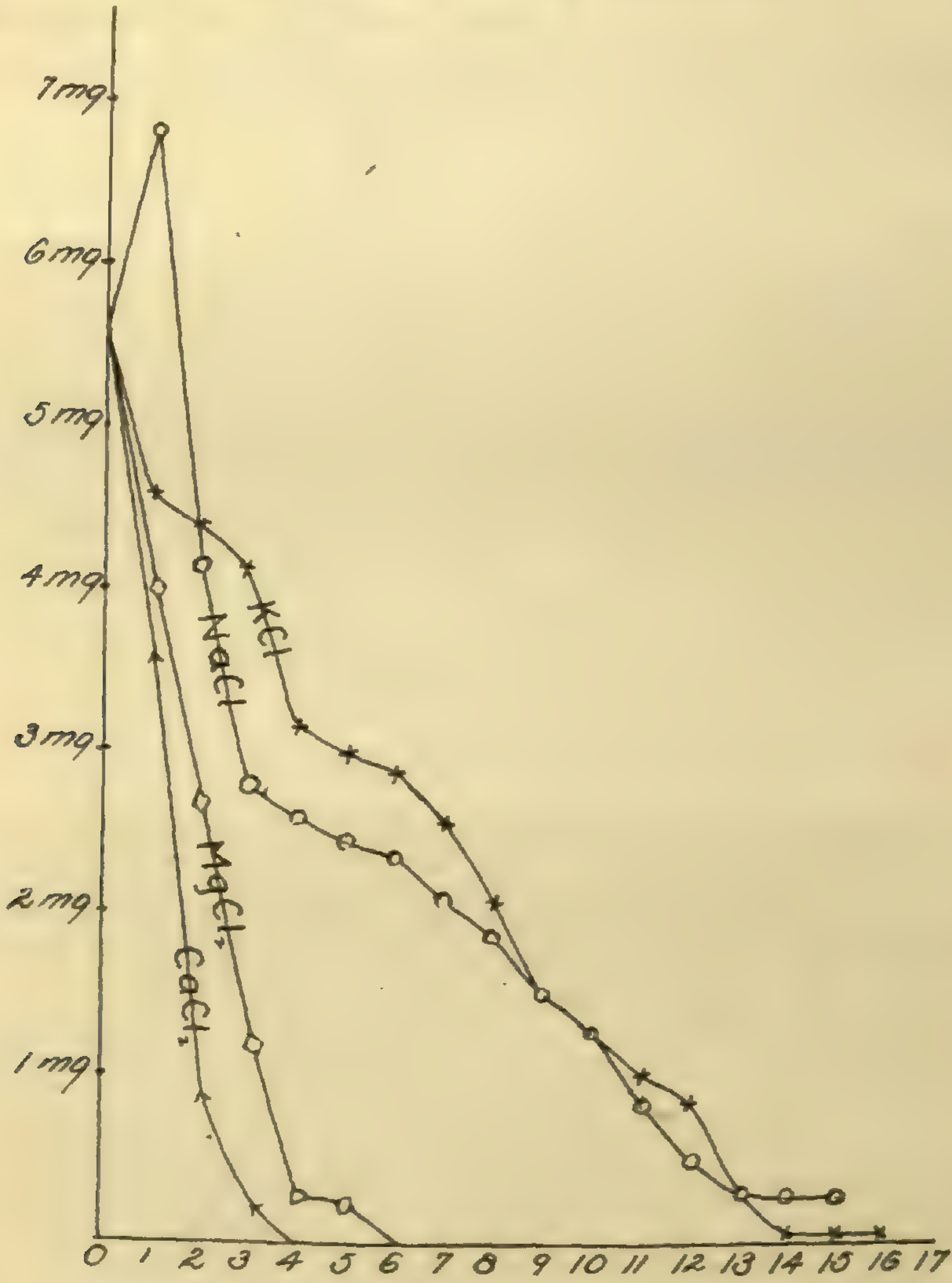

FIG. 1.—Toxicity curves of KCl, NaCl, $MgCl_2$, and $CaCl_2$. The ordinates represent milligrams of ammonia nitrogen. The ordinate at 0 represents the amount of ammonia nitrogen formed in a blank culture (1 per cent. peptone in distilled water). The abscissae represent concentrations in tenths molecular.

SERIES II. CALCIUM CHLORID

Here the experiment was carried out in the same general way as in series I, $CaCl_2$ being substituted for NaCl, and, owing to the extreme toxicity of the former, solutions ranging from 0.1 *m* to 0.6 *m* only were prepared. The ammonia was determined as above and the results are shown in table II.

Here again (see *fig. 1*) we find agreement between the toxic action of $CaCl_2$ on bacteria with that of the same salt and other calcium salts on animals as shown by LOEB (**8**, p. 425) in the case of Fundulus, and by OSTWALD (**21**) in his work on the freshwater Gammarus. It is well to note here also that an examination of the four curves (*fig. 1*) for the salts employed reveals the fact that $CaCl_2$ is easily the most toxic of all for *B. subtilis*. This fact is of especial interest because of its wide disagreement with the facts obtained by experiments on plants with $CaCl_2$, in which MAGOWAN (**14**), for example, found it to be the least toxic of the four salts for wheat (variety Early Genesee). In this respect therefore, if *B. subtilis* may be considered representative, bacteria exhibit the physiological characteristics more typical of animals than of plants, with which they are now classed. We see above, that even at a concentration of 0.3 *m* $CaCl_2$ the formation of ammonia by *B. subtilis* is inhibited.

TABLE II

Numbers represent tenths *m* $CaCl_2$ solution	Milligrams of N formed as NH_3
0	5.60
1	3.15
2	0.91
3	0.21
4	0.00
5	0.00
6	0.00

SERIES III. POTASSIUM CHLORID

The experiment was arranged as those preceding it and the results are shown in table III.

On examining the curve (*fig. 1*) obtained from these results we see at once the strong resemblance of it to that obtained with the solution of NaCl, and although at the concentrations employed KCl exhibits no stimulating effect, it may show it at some concentration lower than 0.1 *m*. This general agreement of the chlorids of K and Na has been found to be even more striking by MAGOWAN (**14**) in a series of experiments on wheat. Here, therefore, *B. subtilis* exhibits physiological characteristics akin to those of the higher plants and differing widely from those of animals, as shown, for

TABLE III

Numbers represent tenths *m* KCl solution	Milligrams of N formed as NH_3
0	5.60
1	4.55
2	4.41
3	4.13
4	3.15
5	3.01
6	2.87
7	2.59
8	2.10
9	1.54
10	1.33
11	0.84
12	0.49
13	0.28
14	0.07
15	0.07
16	0.07

example, by the work of OSTWALD (**21**) in which KCl showed the most extreme toxicity of all the salts tested.

It is also worthy of mention here, as shown above, that the KCl curve declines more gradually than the sodium curve beyond the concentrations of 0.1 *m*.

SERIES IV. MAGNESIUM CHLORID

In the curve drawn on the basis of table IV (*fig. 1*) we notice a parallel to the toxicity curve for $CaCl_2$, except that $MgCl_2$ is not nearly as toxic for *B. subtilis* as the former. While a 0.3 *m* solution of $CaCl_2$ totally inhibits the ammonifying activity of *B. subtilis*, the latter will make a fair growth in a solution of $MgCl_2$ of like concentration and form an appreciable amount of ammonia. Here again, we find agreement between the behavior of *B. subtilis* and Fundulus in solutions of $MgCl_2$, as LOEB (**7,** p. 411) found that in an *m*/2 solution of $MgCl_2$ no embryo develops in the eggs of Fundulus, but that the same degree of toxicity is reached in an *m*/8 solution of $Ca(NO_3)_2$, thus showing the magnesium salt to be less toxic than the calcium salt. On the other hand, as shown by MAGOWAN (**14**), $MgCl_2$ is far the most toxic of the four chlorids employed in experiments on wheat; and in this respect the behavior of *B. subtilis* resembles that of Fundulus rather than that of the higher plants.

TABLE IV

Numbers represent tenths *m* $MgCl_2$ solution	Milligrams of N formed as NH_3
0	5.60
1	4.00
2	2.70
3	1.20
4	0.28
5	0.28
6	0.00

Experiments with binary solutions

The fact that the results obtained were in such striking general agreement with those of the investigators above mentioned on animals and higher plants was sufficient stimulus for further inquiry into the biochemistry of ammonification. It was deemed of interest, therefore, to see if antagonism between salts holds as well for bacteria as it does for the higher forms of life, with the end in view of ascertaining whether balanced solutions are necessary for bacteria, and whether solutions balanced for the other forms of life investigated will prove the same for bacteria. The remainder of this paper will deal with the antagonistic effects of one salt on another in binary solutions, while

the establishment of a balanced solution, with other work now contemplated by the writer, will form the subject of another article.

SERIES V. POTASSIUM CHLORID *vs.* CALCIUM CHLORID

In these experiments the technique was somewhat different from the foregoing. All the salts were used at a concentration of 0.35 m^2 and mixed with each other in various proportions in Erlenmeyer flasks of 250^{cc} capacity. Two or three liters each of KCl and $CaCl_2$ solution of the concentration above noted were made up to contain 0.75 per cent. peptone.[3] In each flask of the first half of the series were placed 100^{cc} of the KCl-peptone solution. To no. 1 nothing was added; to the others up to no. 6 there were added respectively 5, 10, 25, 50, and 100^{cc} of the $CaCl_2$-peptone solution. Then beginning at the other end of the series each flask received 100^{cc} of the $CaCl_2$-peptone solution. Flask no. 11 contained this alone; to the preceding ones in order were added respectively 5, 10, 25, 50, and 100^{cc} KCl-peptone solution, the two halves of the series meeting in no. 6, as shown in the curve (*fig. 2*) and table V, at the combination of 100^{cc} of each solution. In order to keep the volume the same in all cultures they were thoroughly mixed, and enough solution drawn off with the pipette, where additions were made, to make all the culture

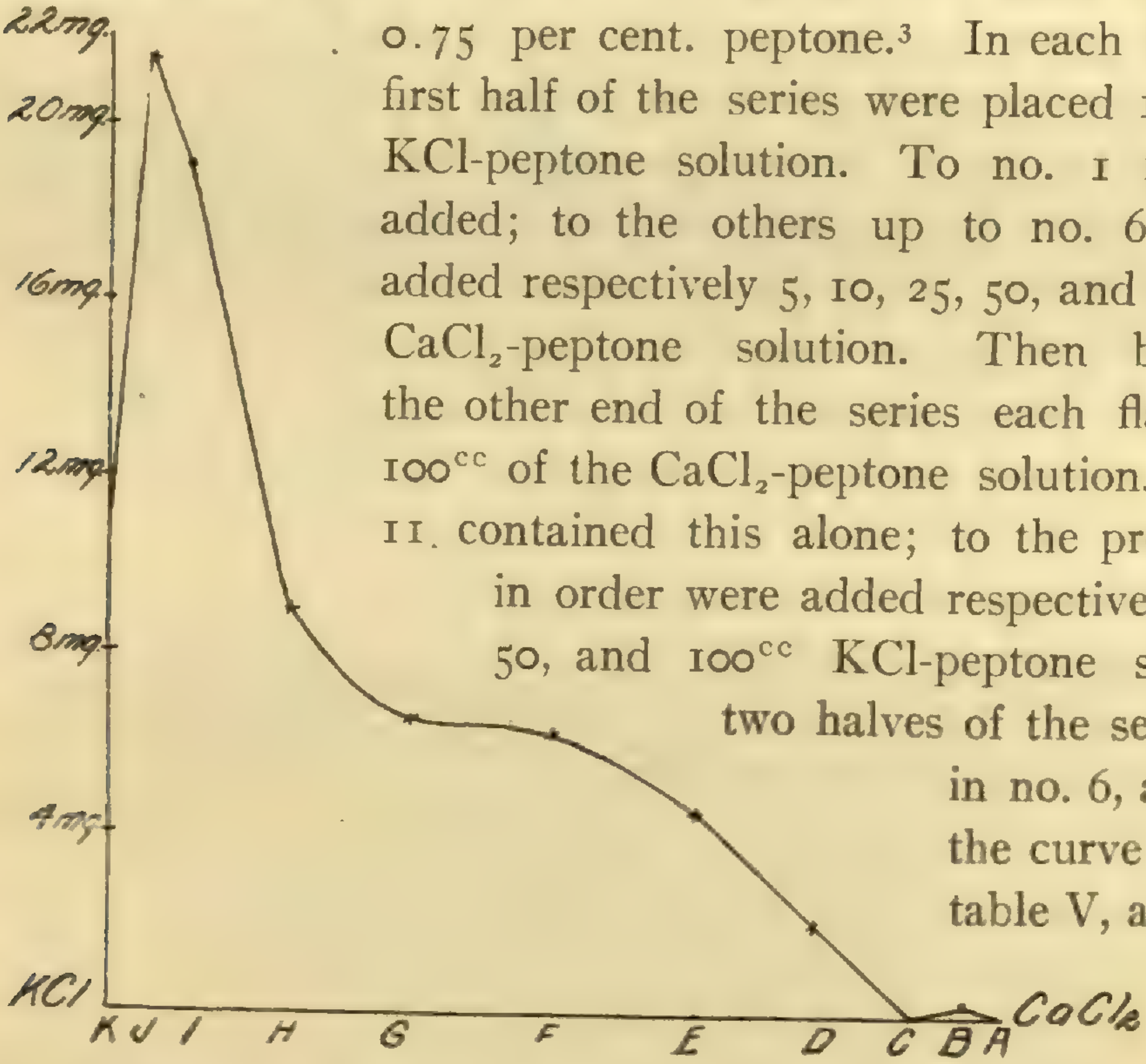

FIG. 2.—Antagonism curve, KCl *vs.* $CaCl_2$. The ordinate at K represents the ammonia nitrogen in milligrams formed in a pure KCl solution. The ordinate at A represents the amount of ammonia nitrogen formed in a pure $CaCl_2$ solution, and the ordinates at the intermediate points represent the amounts formed in various combinations of the two salts as indicated by the corresponding letters in table V.

[2] This concentration was chosen because it was about the concentration of NaCl in sea water of San Francisco Bay.

[3] This concentration of peptone was used in all the experiments with binary solutions.

solutions have a uniform bulk of 100^{cc}, thus avoiding any differences in the supply of oxygen to the bacteria by having an equal surface of liquid exposed to the air in each flask.

As in the case of the single salt solutions, duplicates were run on all the cultures, and also sterile controls, so as to allow of a determination of the ammonia actually formed by the bacteria. The solutions were all sterilized in the autoclave at 1.25 atmospheres, inoculated as above, and incubated for two and a half days at 28° to 29° C., after which they were distilled, as in the case of the single salt cultures, and the ammonia determined. The results obtained follow, with the curve plotted from them in accordance with the arrange-

TABLE V

ALL QUANTITIES GIVEN REFER TO CUBIC CENTIMETERS OF 0.35 *m* SOLUTIONS

Culture solution	Corresponding points on curve	Milligrams of N formed as NH_3
100 KCl	K	10.92
100 KCl, 5 $CaCl_2$	J	21.46
100 KCl, 10 $CaCl_2$	I	18.83
100 KCl, 25 $CaCl_2$	H	9.00
100 KCl, 50 $CaCl_2$	G	6.44
100 KCl, 100 $CaCl_2$	F	6.30
50 KCl, 100 $CaCl_2$	E	4.55
25 KCl, 100 $CaCl_2$	D	0.25
10 KCl, 100 $CaCl_2$	C	0.14
5 KCl, 100 $CaCl_2$	B	0.25
100 $CaCl_2$	A	0.00

ment employed by OSTERHOUT (**19**). The letters along the axis of abscissas represent a given combination of the two salts as indicated in the table, and the ammonia formed is laid off on the axis of ordinates in numbers representing milligrams.

We see at a glance (*fig. 2*) that there is a strong antagonism

between $CaCl_2$ and KCl. OSTERHOUT (**20**) obtained a similar curve for the antagonism between the same two salts in his experiments on wheat. It is particularly interesting to note that both for wheat and for bacteria the maximum point of the curve is at the combination of 100^{cc} KCl solution and 5^{cc} $CaCl_2$ solution, notwithstanding the wide difference between the materials employed in the two cases, and further despite the fact that the Ca ions are most toxic for *B. subtilis* and least toxic for the wheat.

Again we find the strong antagonism above obtained is in striking accord with the results of LOEB in similar experiments on animals, both as to development (**7**) and as to muscular contraction (**6**).

SERIES VI. SODIUM CHLORID *vs.* MAGNESIUM CHLORID

In this series the experiment was arranged and carried out in the same manner as the one preceding, except that the salts used were different. The ammonia formed at the end of the period of incubation was determined with the following results:

TABLE VI

ALL QUANTITIES GIVEN REFER TO CUBIC CENTIMETERS OF 0.35 *m* SOLUTIONS

Culture solution	Corresponding points on curve	Milligrams of N formed as NH_3
100 NaCl	K	22.36
100 NaCl + 5 $MgCl_2$	J	23.75
100 NaCl + 10 $MgCl_2$	I	28.67
100 NaCl + 25 $MgCl_2$	H	24.56
100 NaCl + 50 $MgCl_2$	G	18.67
100 NaCl + 100 $MgCl_2$	F	10.49
50 NaCl + 100 $MgCl_2$	E	4.84
25 NaCl + 100 $MgCl_2$	D	3.80
10 NaCl + 100 $MgCl_2$	C	3.54
5 NaCl + 100 $MgCl_2$	B	3.36
100 $MgCl_2$	A	2.13

In the curve plotted from table VI (*fig. 3*), we note again a strong antagonism between the two salts tested, though it is not as marked as in the last series. The curve proves to be more regular than the last, probably owing to the fact that there was practically no variation in the temperature throughout the period of incubation. As LIP-

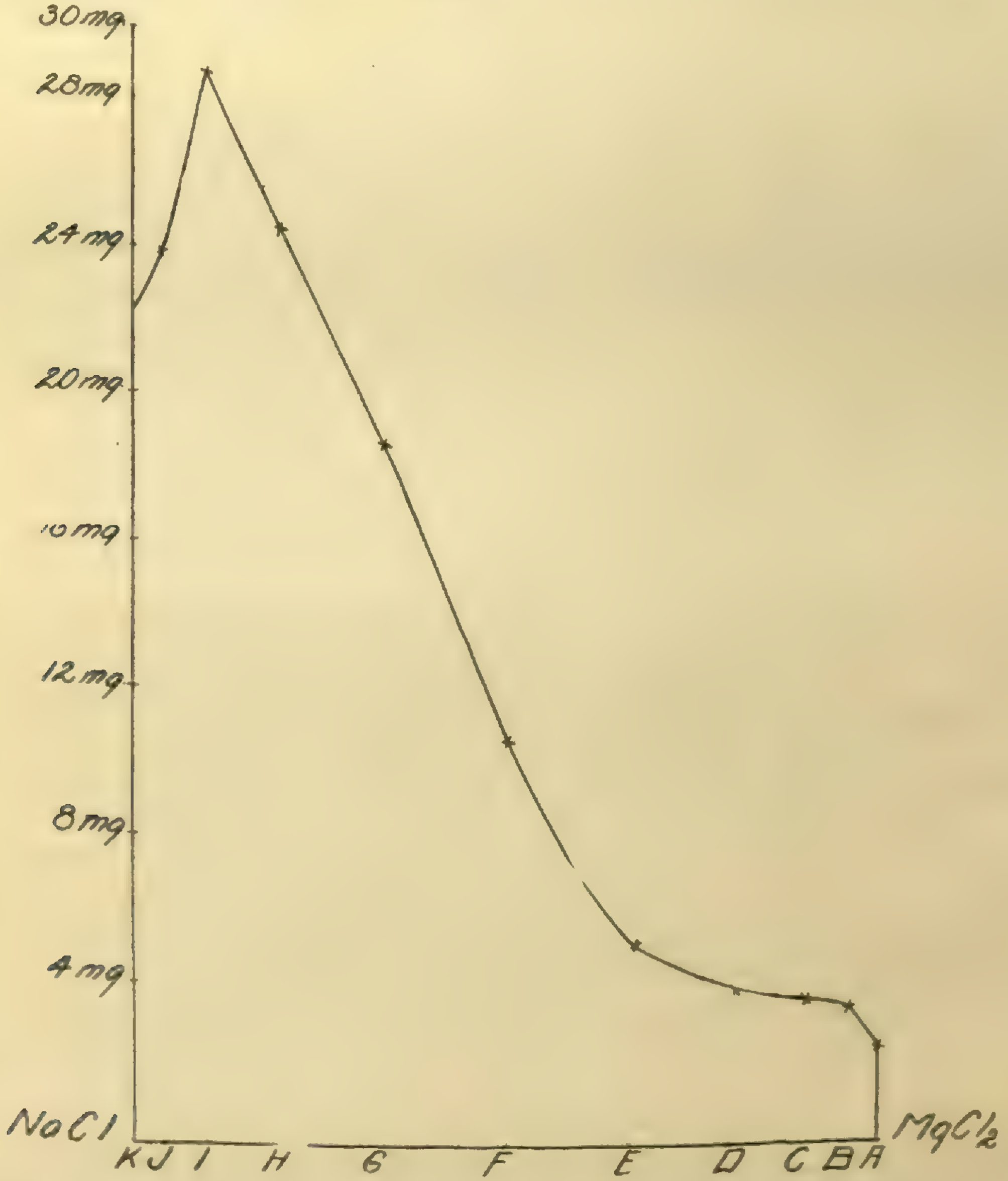

FIG. 3.—Antagonism curve, NaCl *vs.* $MgCl_2$. The ordinate at K represents the ammonia nitrogen in milligrams formed in a pure NaCl solution. The ordinate at A represents the amount of ammonia nitrogen formed in a pure $MgCl_2$ solution, and the ordinates at the intermediate points represent the amounts formed in various combinations of the two salts as indicated by the corresponding letters in table VI.

MAN (4) has demonstrated, a constant temperature and equal periods of incubation are essential factors in quantitative work in ammonification, if results are to be considered comparable.

It is a significant fact that here again the maximum point on the curve nearly coincides with that of a similar curve obtained by OSTERHOUT (**19**) in his experiments with the same salts on root development in wheat, and exactly coincides in the case of a fungus (*Botrytis cinerea*); and though OSTERHOUT employed such widely varying concentrations as 0.12 *m* in the case of the wheat and 1.5 *m* in Botrytis, the maximum development was reached in a mixture of 10^{cc} of the $MgCl_2$ solution (or 7.5^{cc} for wheat) and 100^{cc} of the NaCl solution, just as was the case in ammonification by *B. subtilis*.

In his experiments on the eggs of Fundulus and the sea-urchin (Arbacia), LOEB (**10**) found that in a mixture of 98^{cc} 5*n*/8 NaCl and 2^{cc} 10*n*/8 $MgCl_2$ all the eggs of Fundulus form embryos, whereas in pure NaCl or $MgCl_2$ solutions alone no embryos would form, and even in a mixture of equal parts of the above-mentioned solutions 75 per cent. of the eggs formed embryos. On the other hand, OSTWALD (**21**) found in his work on the freshwater Gammarus that, so far from exercising an antagonistic effect on each other, the combination of Mg and Na chlorids proved more poisonous than either alone.

SERIES VII. MAGNESIUM CHLORID *vs.* CALCIUM CHLORID

The arrangement of the experiment and the ammonia determinations were carried out in a manner similar to that employed in the two preceding series, two bivalent salts being tested this time. The results were as shown in table VII, p. 117.

By an examination of the curve drawn on the basis of table VII (*fig. 4*) we are confronted by the very striking instance of lack of antagonism between the two salts. On the contrary, there is a constant increase of the toxic properties of each when the other is added to it in increasing amounts. In this exceptional behavior, so far as the writer can ascertain, *B. subtilis* (and probably all the ammonifiers) stand alone, when their physiological efficiency in such salt mixtures is compared with that of the higher plants and animals. No instance of such behavior on the part of any member of the latter two groups of organisms has come to my notice in reviewing the results of similar researches.

An antagonism between $CaCl_2$ and $MgCl_2$, though slight, was

found to be none the less definite by LOEB (**6**) in experiments which showed that sea-urchin blastulae and gastrulae would swim about

TABLE VII

ALL QUANTITIES GIVEN REFER TO CUBIC CENTIMETERS OF 0.35 *m* SOLUTIONS

Culture solution	Corresponding points on curve	Milligrams of N formed as NH_3
100 $MgCl_2$	K	3.08
100 $MgCl_2$ } 5 $CaCl_2$ }	J	2.59
100 $MgCl_2$ } 10 $CaCl_2$ }	I	1.68
100 $MgCl_2$ } 25 $CaCl_2$ }	H	.98
100 $MgCl_2$ } 50 $CaCl_2$ }	G	.21
100 $MgCl_2$ } 100 $CaCl_2$ }	F	.07
50 $MgCl_2$ } 100 $CaCl_2$ }	E	.00
25 $MgCl_2$ } 100 $CaCl_2$ }	D	.00
10 $MgCl_2$ } 100 $CaCl_2$ }	C	.00
5 $MgCl_2$ } 100 $CaCl_2$ }	B	.00
100 $CaCl_2$	A	.49

in a mixture of the salts above mentioned for forty-eight hours, while each salt by itself would immediately prove poisonous at the concen-

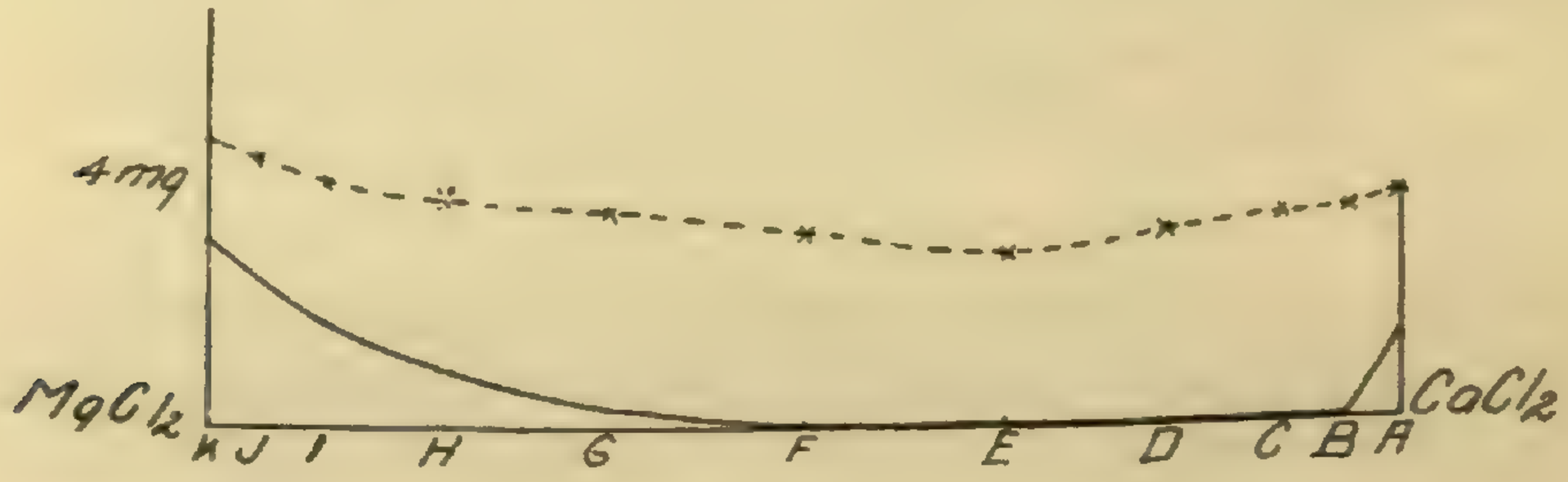

FIG. 4.—Non-antagonism curves, $CaCl_2$ *vs.* $MgCl_2$. The ordinates at K represent the ammonia nitrogen in milligrams formed in a pure $MgCl_2$ solution. The ordinate at A represents the amount of ammonia nitrogen formed in a pure $CaCl_2$ solution, and the ordinates at the intermediate points represent the amounts formed in various combinations of the two salts as indicated by the corresponding letters in tables VII (unbroken line) and VIII (dotted line).

tration employed in the combination. Another interesting case in point may be noted in the experiments of the same investigator on Polyorchis (**11**), a jelly-fish of San Francisco Bay. In a solution of 50^{cc} NaCl + 6^{cc} $MgCl_2$ + 1^{cc} $CaCl_2$, the rhythmical contractions of the margin go on normally, but with a slight increase of $CaCl_2$ the contractions are inhibited, and when 5^{cc} of a $3m/8$ solution of $CaCl_2$ are added, they are completely suppressed. On the other hand, when the margin of the fish, containing the sense organs and the central nervous system, is cut off, $CaCl_2$ exercises a stimulating action on the isolated center of the fish, and contractions will go on normally; but when $MgCl_2$ is added to the solution, in the ratio of 4 parts $MgCl_2$ to 1 part $CaCl_2$, the stimulating action of the $CaCl_2$ is suppressed and contractions cease. In both cases, therefore, there is evidence of a definite antagonism between Ca and Mg. Likewise, LILLIE (**3**) proved the existence of antagonism between the two salts, when he found that the ciliary activity of the larvae of Arenicola would go on normally for some time in a mixture of approximately 4 parts $MgCl_2$ to 1 part $CaCl_2$, whereas it would immediately cease if either of the salts at the same concentration was present alone.

Again, we find the well-known researches of LOEW and his pupils (**12, 13**), and later the researches of KEARNEY and CAMERON (**1**), which show in the higher plants the strong antagonism between calcium and magnesium. The last-named investigators found, in their experiments with the white lupin (*Lupinus albus*) and with alfalfa (*Medicago sativa*), that when $CaCl_2$ was added to $MgSO_4$ in about equal proportions, the plants exhibited about 160 times the tolerance for the latter salt that they did in solutions of $MgSO_4$ alone. They found, further, that the antagonism between $CaCl_2$ and $MgCl_2$, though not so great (increasing the tolerance about 40 times), was nevertheless very marked, and where $CaSO_4$ replaced $CaCl_2$ the antagonism was very much greater between Ca and Mg than in either of the cases above cited.

I wish to cite only one more case, which emphasizes by strong contrast the exceptional results obtained above in experiments with *B. subtilis;* that is, the results of highly ingenious experiments on rabbits and a monkey by MELTZER and AUER (**16**) showing the antagonistic effect of calcium on the inhibitory effect of magnesium. As a

typical instance may be cited experiment 1 of their series, in which about 13^{cc} of an $m/1$ solution of $MgCl_2$ was injected subcutaneously. Less than half an hour later there was produced general anaesthesia, with all the attending symptoms. When 2^{cc} of a solution of $m/8$ $CaCl_2$ was injected into the ear vein the rabbit was again breathing normally, and when 8^{cc} had been given the animal sat up and appeared entirely recovered, except for a stiffness in the hind legs.

In these experiments, some of which were even more striking than the one cited, MELTZER and AUER employed, besides the chlorids of Ca and Mg, the acetate and nitrate of the former, and the acetate, nitrate, and sulfate of the latter. The same strong antagonism was noted in all cases.

TABLE VIII

ALL QUANTITIES GIVEN REFER TO CUBIC CENTIMETERS OF 0.35 m SOLUTIONS

Culture solution	Corresponding points on curve	Milligrams of N formed as NH_3
100 $MgCl_2$	K	4.76
100 $MgCl_2$ + 5 $CaCl_2$	J	4.48
100 $MgCl_2$ + 10 $CaCl_2$	I	4.20
100 $MgCl_2$ + 25 $CaCl_2$	H	3.78
100 $MgCl_2$ + 50 $CaCl_2$	G	3.64
100 $MgCl_2$ + 100 $CaCl_2$	F	3.22
50 $MgCl_2$ + 100 $CaCl_2$	E	3.08
25 $MgCl_2$ + 100 $CaCl_2$	D	3.29
10 $MgCl_2$ + 100 $CaCl_2$	C	3.43
5 $MgCl_2$ + 100 $CaCl_2$	B	3.57
100 $CaCl_2$	A	3.78

In addition to the confirmation of the results stated above in experiments with the same material, one series was also carried out with a culture of *B. subtilis* obtained from New Jersey, and with salt solutions made up from a different grade of chemically pure salt.

As can be seen from the following table and also from *fig. 4*, the results fully confirm those above given; and though the absolute amounts are different, the results are relatively the same.

It may be of interest to note here that *B. subtilis* from a 24-hour peptone agar slope was examined in hanging drops of molecular solutions of magnesium chlorid and calcium chlorid, and the organisms showed no perceptible ill effects from the action of the solution. The ciliary movements appeared normal even after 24 hours in the hanging drop. It was noticed, however, that there was little or no division during the 24 hours and it is likely that the calcium and magnesium salts exercise their toxic effects, partly at least, by inhibiting reproduction, since the ciliary movements seemed to go on without interruption. These remarks, however, are based on too meager experimental evidence to be anything else than conjecture at present, but they serve to indicate a field of most interesting research.

Though they are not analogous to the lack of antagonism between Ca and Mg shown above, it is interesting to note two cases on record, in which the addition of one salt to another made a combination more toxic than either. One case is that cited above from OSTWALD'S experiments on the freshwater Gammarus, in which a combination of $MgCl_2$ and NaCl in solution was more toxic to that animal than either of these in solution alone. The other case is that noted in the experiments of KRÖNIG and PAUL (**2**), who found that the value of mercuric sulfate, acetate, and nitrate as disinfectants was enhanced by the addition of small amounts of the chlorids of the alkalies (K and Na); but, on the other hand, that the addition of the same chlorids to $HgCl_2$ reduced considerably the disinfecting powers of the latter.

The first instance is not analogous to the results of the writer, because one of the salts used was different, and the experiment was carried out under conditions so totally different that the value of a comparison is doubtful. In the second instance, as KRÖNIG and PAUL themselves suggest in the same article, the increase of toxicity is not necessarily owing to a lack of antagonism between the two salts, but rather to the formation of complex double salts of mercury, which are characteristic of that element, and therefore this again cannot be compared with the lack of antagonism between Ca and Mg above noted.

SERIES VIII. SODIUM CHLORID *vs.* POTASSIUM CHLORID

This series was carried out to determine the antagonistic action between KCl and NaCl and was arranged in the same manner as the foregoing series. The ammonia determinations gave the following results:

TABLE IX

ALL QUANTITIES GIVEN REFER TO CUBIC CENTIMETERS OF 0.35 *m* SOLUTIONS

Culture solution	Corresponding points on curve	Milligrams of N formed as NH_3
100 NaCl	K	10.99
100 NaCl + 5 KCl	J	11.62
100 NaCl + 10 KCl	I	10.50
100 NaCl + 25 KCl	H	10.08
100 NaCl + 50 KCl	G	9.66
100 NaCl + 100 KCl	F	11.62
50 NaCl + 100 KCl	E	17.57
25 NaCl + 100 KCl	D	14.42
10 NaCl + 100 KCl	C	13.44
5 NaCl + 100 KCl	B	11.41
100 KCl	A	10.57

That the like valences of the two salts employed do not prevent antagonistic action in the case of *B. subtilis* (as may have been surmised from results in the last series) can be seen from the curve in *fig. 5*. Here again, there is a marked resemblance between the effect of this combination of metallic salts on *B. subtilis* and on animals and higher plants. LILLIE, for example, showed (**3**) that the ciliary activity of the larvae of Arenicola, which was inhibited in solutions of either KCl or NaCl alone, went on normally when solutions of the two at the same concentrations were mixed in the proportion of 20 parts NaCl to 8 parts KCl.

LOEB (**9**) and OSTWALD (**22**) also found, in working on a marine

and a freshwater Gammarus respectively, that a distinct antagonism exists between NaCl and KCl. Among plants we find that the work of OSTERHOUT (**20**) also shows some antagonism between KCl and NaCl, and here again it is significant to note that there are two maxima.

The curve in *fig. 5* shows an unusually gradual rise and decline.

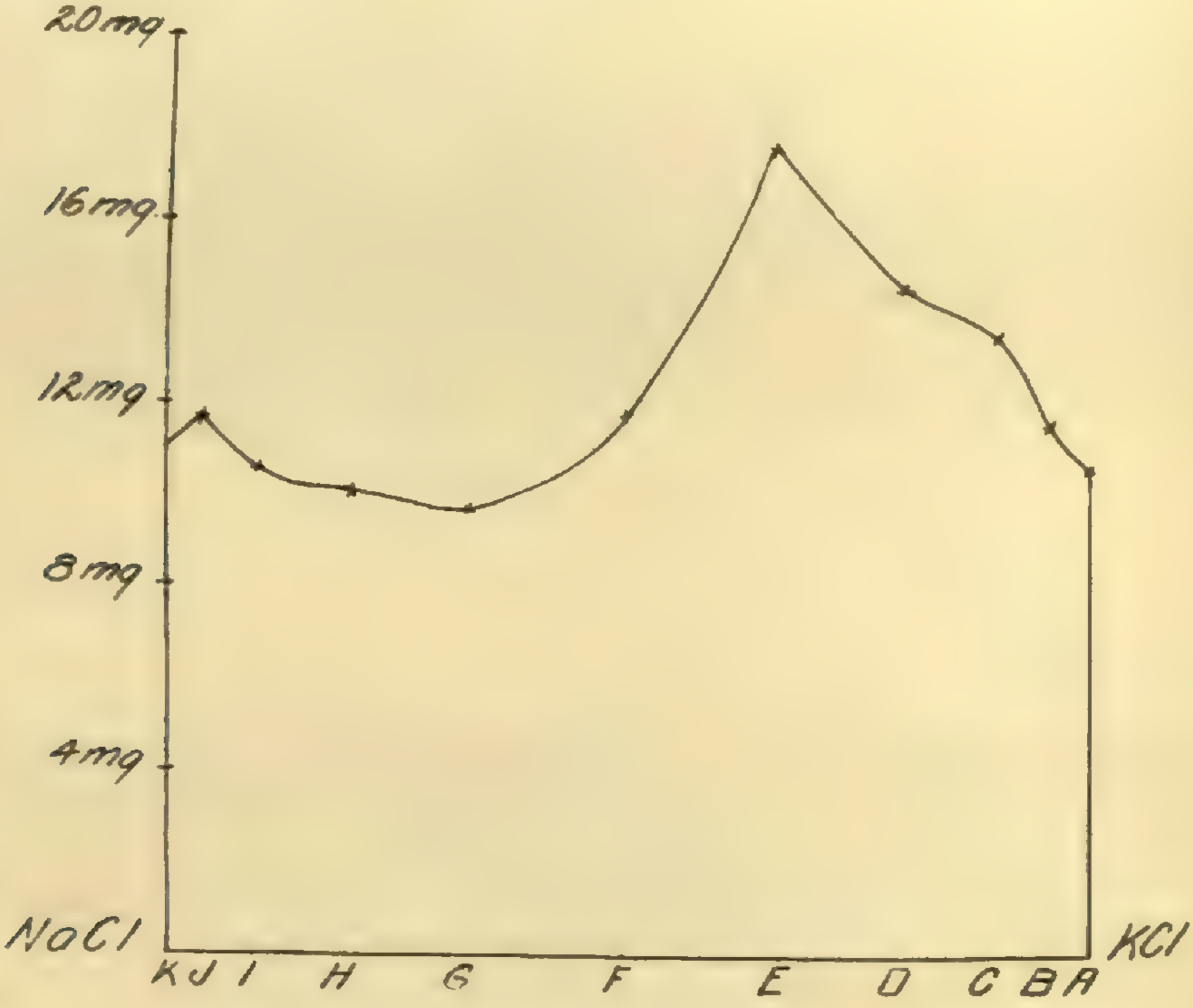

FIG. 5.—Antagonism curve, NaCl *vs.* KCl. The ordinates at K represent the ammonia nitrogen in milligrams formed in a pure NaCl solution. The ordinate at A represents the amount of ammonia nitrogen formed in a pure KCl solution, and the ordinates at the intermediate points represent the amounts formed in various combinations of the two salts as indicated by the corresponding letters in table IX.

This is characteristic of the Na and K chlorids used singly, where they become gradually more and more toxic with the increase of concentration. It is consequently in strong contrast to the sharp decline of the calcium and magnesium curves, especially that of the former. In this curve, as above noted, there are two maximal points, one at the combination of 100^{cc} of NaCl + 5^{cc} KCl, and another at the combination of 100^{cc} KCl + 50^{cc} NaCl. In three confirmatory series on this experiment, the maximal points were in each case obtained at the same combinations.

Discussion

Briefly reviewing the results with *B. subtilis* above given, we note their significance from the scientific as well as the practical standpoint. Of the four chlorids tested singly, NaCl is the only one stimulating ammonification at the concentrations employed. It is not unlikely, however, that KCl has a similar effect at a slightly lower concentration.

$CaCl_2$ is the most toxic of the chlorids used. In this feature, *B. subtilis* appears to resemble animals, for which calcium is very toxic, and not plants; since for plants, with which bacteria are now classed, calcium is the least toxic of the four chlorids. This fact may have a bearing on the future classification of bacteria.

The strong antagonism exhibited in some of the combinations of salts employed speaks eloquently for the fact that balanced solutions are as necessary for the optimal development of bacteria and allied forms as for the higher plants and animals, a fact which has been denied in a recent publication of LOEW and ASO (**13**). This is of the greatest practical significance when applied to soil bacteria, and especially those of alkali soils, in which, owing to the excessive amount of one or more salts, the bacterial activity is inhibited, and consequently plant food is not made available to the higher plants.

Because the salts experimented with are all found in our soils in larger or smaller amounts, and in some soils are present in excess, it is a matter of practical importance to apply the results of researches on the physiology of plants and bacteria to improve such alkali lands. There is no doubt in my mind that when we have learned to coordinate the results of researches on plants and soil bacteria, and to apply them in the field, we shall have at our command a method for the control and profitable cultivation of alkali lands, of which so many thousands of acres are merely vast wastes at the present time.

Lastly, the results of these experiments are significant because they open up an unexplored field of bacterial physiology, in which further researches will teach us much.

Summary

1. Each of the four chlorids ($CaCl_2$, $MgCl_2$, KCl, NaCl) is toxic for *B. subtilis*, in the order given, the first being the most toxic and

the fourth the least. This is quite different from the results with higher plants, where magnesium is the most toxic and calcium the least.

2. A marked antagonism exists between Ca and K, Mg and Na, K and Na.

3. No antagonism exists between Mg and Ca, but the toxic effect of each is increased by addition of the other to it. This is just the opposite of what has hitherto been found for plants.

In conclusion, the writer desires to express his indebtedness to Prof. W. J. V. OSTERHOUT, at whose instance these researches were begun, for helpful suggestions and kindly criticism throughout the course of the investigations.

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THE GAMETOPHYTES OF CALOPOGON

LULA PACE

(WITH PLATES VII–IX)

Photolith plates 2nd edition

This paper reports a continuation of the work on certain orchids begun in 1906. The peculiarities in the development of the megaspores, and in the number and origin of the nuclei in the embryo sac found in Cypripedium (14) made it seem desirable to continue the investigation.

The same methods were used as in the work on Cypripedium. The material of *Calopogon pulchellus* R. Br. was collected near Chicago in the summer of 1906. The usual chromacetic and alcohol-formalin solutions were carried to the field and the material was killed as collected. The greater part of it has been cut in serial sections five to seven microns thick, and stained in safranin and gentian violet.

MEGASPORES

The ovules are very numerous and even smaller than those of Cypripedium, the mother cell being five to ten microns in diameter. The archesporium does not seem to be differentiated early. *Fig. 1* shows an ovule pretty well advanced, and yet it is not possible to distinguish an archesporial cell by any difference in staining. The one drawn shows the greatest difference in size in favor of the usual archesporial cell, but other ovules in the same ovary show the other cells of the axial row to be larger; so here also it is probably the cell that is approaching mitosis that is larger. *Fig. 2* is taken from another ovary in which the ovules are apparently in the same stage as in *fig. 1*. This one suggests a more advanced stage by the broadening of the ovule. Yet this may be related to the appearance of more than the one mother cell, for these ovules with two sporogenous cells are broader, as will be seen later. The first undoubted archesporium seen was in the stage shown in *fig. 3*. Here the ovule is much larger than in the first two figures and the first integument is beginning. In this ovary more than half of the ovules were in this stage, the archesporial cell being large and showing difference in staining reaction.

The other ovules were in the mother cell stage (*fig. 4*). The integument is only a trifle more advanced in the latter than in the former, but the nuclei are in all stages of synapsis. The archesporial cell may become the mother cell without division, and no parietal cell was ever found. But it is possible that in some cases it divides; for an archesporium of more than one cell was rare, being found only once (*fig. 26*), and yet several ovules with two mother cells in position to have been formed by division of a single archesporial cell were found (*figs. 27, 29*). It is evident that these must have originated by a division of the archesporial cell, or the archesporium must have consisted of two cells.

A more remarkable case still is that in which two distinct sporogenous areas are differentiated in the same ovule (*figs. 28–31*). *Fig. 31* shows several cells of nucellar tissue between the two mother cells, the cut being across the ovule in a different direction from that of *fig. 28*. A somewhat later stage, similar to *fig. 28*, is shown in *fig. 30*. *Fig. 32* shows at least one of the mother cells divided, and one of the resulting daughter cells increasing for the second division, the other disintegrating in the usual fashion. The presence of more than one mother cell is not rare in my material. Of about sixty ovaries cut, thirteen showed ovules with two mother cells, or stages derived from this condition. In these thirteen ovaries, the ovules showing this condition vary from one to seven, giving a total of thirty-seven. Of these thirty-seven, twenty-one are similar to *figs. 28* and *31*, and sixteen resemble *figs. 27* and *29*. There was in all probability double this number, for only those were counted in which both cells appeared in the same section. No attempt was made to trace the ovules from section to section for this condition, and it is evident that there are more chances against getting both in the same section than in favor of it. *Fig. 31* is a drawing from a single section, but the whole ovule was traced carefully from section to section for evidence of the coalescence of two ovules. The only abnormal appearance was the unusually broad funiculus. *Fig. 33* shows the megaspores derived from the two adjacent mother cells. In each case the chalazal megaspore is developing and the other three are disintegrating. In the lower or micropylar group the megaspores are in a row—the usual row of four, except that the wall is lacking between the two upper megaspore

nuclei. In the upper or chalazal group two of them lie side by side without a separating wall. The upper two are in the normal position, but the usual separating wall is lacking. *Fig. 34* seems to show an embryo sac of two nuclei derived from the chalazal group of two mother cells. The three disintegrating nuclei are probably megaspore nuclei, and the micropylar mother cell has not divided. *Fig. 35* is taken from a much younger ovule, but a very broad one. It seems to be the product of two sporogenous cells, the chalazal mother cell having divided to form the two daughter nuclei, the micropylar mother cell being still in metaphase. It is possible that this is the result of the second division of the chalazal daughter cell without walls separating the two megaspore nuclei, the micropylar daughter cell being still in mitosis. But the chromosomes in this nucleus give every appearance of the heterotypic division, and the ovule is so unusual in appearance that it seems to have had two mother cells.

Synapsis continues for some time, if the extent of development of the integument be considered proof (*figs. 4, 5*). The division of the mother cell takes place in the usual way (*figs. 5–10*), giving the two daughter cells (*fig. 11*). The number of chromosomes is apparently thirteen (*fig. 6*), although only a few counts were made, but this one seemed unusually distinct, so it is probably the correct number. Several counts were attempted in a sporophytic area, giving approximately twenty-six. It is probable that sometimes the wall separating the daughter nuclei fails to appear. *Fig. 9* would be expected to show some evidence of wall formation, if it were going to appear. *Fig. 15* shows the division completed, with no suggestion of a wall. *Fig. 16* gives no wall. Several examples of this failure of the wall were found, and in others it seemed very faint and probably disappeared.

In many plants the chalazal daughter cell divides; but in Calopogon it seems quite common for both to divide, although the micropylar cell is even then somewhat smaller than the chalazal cell (*figs. 13, 14*). These divisions may occur at the same time as in the above figures, or either may precede the other. *Figs. 17* and *18* show the chalazal cell dividing first, the former showing the micropylar cell somewhat more advanced than the latter. In *fig. 19* the micropylar daughter cell has almost completed the division, while the chalazal

cell is still in the spirem stage. This does not appear so often as the other in my material. At this division of the daughter cells the walls begin to form in the usual way (*figs. 14, 18, 19*), though in a few instances no evidence of wall formation was seen (*figs. 17, 20*). But the walls all disappear, if they were ever found in these cells (*figs. 20, 28*), for no case of a wall at this stage was seen, though hundreds of examples like those figured were found. After this division the micropylar nucleus from the inner daughter cell begins to disintegrate (*figs. 21, 22*), although it may sometimes divide (*fig. 24*). *Fig. 23* shows both daughter cells divided, with one megaspore nucleus in each case disintegrating. While apparently two of the megaspores are active, one of them is already in advance of the other, judging by size of nucleus and cell. *Fig. 24* shows the micropylar daughter cell with the nucleus in metaphase, while *fig. 25* is only late spirem. Probably the former would have completed the division. *Figs. 24* and *25* are also interesting as the only cases seen in which the megaspore nucleus that usually disintegrates in the sac shows evidence of further development. In the first it has divided, while in the second it is in the spirem stage, yet in both cases it is evidently disintegrating. The megaspore that is to form the sac in *fig. 25* is in mitosis with the spindle well formed.

EMBRYO SAC

As the walls disappear, or never develop, in the division of the daughter cells, the two megaspore nuclei are left in the embryo sac. The micropylar nucleus apparently always disintegrates, as does the cytoplasm about this nucleus (*figs. 21, 22, 23, 48*). This leaves only one megaspore nucleus, probably one might say only one megaspore, to enter into the organization of the embryo sac. This nucleus divides, giving a sac with two nuclei (*fig. 46*). The three other bodies in the sac are probably the three disintegrating megaspore nuclei. The two nuclei of the sac divide simultaneously (*fig. 47*), giving a four-nucleate sac (*fig. 48*). This figure is interesting because the three disintegrating megaspore nuclei are easily identified at this late stage. *Fig. 49* shows about the same stage, except that the sac has increased in size and the nuclei are preparing for the next division, although there are still traces of the spindles of the preceding

mitosis. These spindles show no traces of wall formation. The micropylar daughter cell did not divide here, although the nucleus completed the prophase, even forming the chromosomes. There is just a trace of the disintegrating megaspore nucleus in the sac. The four nuclei in the sac increase in size and divide in the usual fashion (*fig. 50*). The two dark bodies in the lower part of this sac are probably the remains of the micropylar daughter cell and the disintegrating megaspore nucleus. This division gives eight nuclei in the sac (*fig. 51*). By comparing *figs. 51* and *52* it can be seen that one of the four nuclei at the lower end passes up toward the center, and the sister to the egg moves down in the usual position for the two polar nuclei. The eight nuclei arrange themselves in the usual way—an egg apparatus of two synergids and the egg in the micropylar end of the sac, three antipodals in the chalazal end, and the two polars near the center (*figs. 52, 53*). *Fig. 53* shows a pollen tube already forcing its way between the integuments, about halfway to the sac.

MICROSPORES AND MALE GAMETOPHYTE

The pollen in Calopogon is in masses or pollinia, as it is in many of the orchids. Each massula is apparently the group of cells resulting from the division of each sporogenous cell (*fig. 36*). The whole sporogenous area probably reaches the mother cell stage at the same time (*fig. 37*). The mother cells divide in the usual way, by the so-called simultáneous division, which is thought to be more characteristic of dicotyledons than of monocotyledons, although found in both groups (COULTER and CHAMBERLAIN **7,** p. 121). This gives the tetrad form in some cases, but in others the four spores lie in any plane (*figs. 38–42*). The microspore nucleus divides into the tube and generative nuclei while the pollen is still in the sac (*fig. 43*). Pollen tubes are numerous in the ovary, in some cases at least, when ovules are still in the mother cell stage, like *fig. 5*, although no pollen tube was ever seen in contact with an ovule at this stage. *Figs. 41* and *42* are examples of pollen tubes within the ovary, before entering the ovules. In these the generative nucleus had already divided into the two male nuclei, so it is probable that this division takes place when the pollen grain germinates.

FERTILIZATION

As has been said, the embryo sac when ready for fertilization contains eight nuclei, arranged in the usual fashion—the egg apparatus of two synergids and an egg in the micropylar end, three antipodals at the opposite end, and the two polars about the center of the sac (*fig. 53*). When the pollen tube is entering the sac, the egg and the polar nuclei may be in late spirem stage (*fig. 54*). In *fig. 55* the two polars have already formed chromosomes. *Fig. 56* shows the fusion of a male nucleus with the egg, and the triple fusion in the center of the sac is undoubtedly that of the two polars and the second male nucleus. Not very much material was examined at this stage. But in all that was seen there was every indication that this is the usual condition.

DISCUSSION

Sporogenous cells.—BOWER (**3**) states that a multicellular archesporium is found in several of the archichlamydeous dicotyledons, especially in Amentiferae, Rananculaceae, Rosaceae; but that it is apparently rare in more advanced dicotyledons and in the monocotyledons. GUIGNARD (**9**) reports *Ornithogalum pyrenaicum* with an archesporium of two cells, only one of which gets beyond the archesporial stage. BARNARD (**1**) reports two embryo sacs in *Lilium candidum*. COULTER and CHAMBERLAIN (**7**, p. 61) say that they have seen one preparation of *Lilium philadelphicum* with three archesporial cells and another with five; but no figures are given. FERGUSON (**8**) in a note figures two mother cells in *Lilium longiflorum* with intervening nucellar tissue. But, as has already been shown, there are many such cases in Calopogon, thirty-seven cases of two mother cells or stages evidently derived from them being seen, in thirteen ovaries out of about sixty cut, and this probably represents less than half the number actually present. So while this condition is very far from being the usual one in my material, it could hardly be called rare, and therefore abnormal. It seems best to regard it as a primitive character that has been retained, or at least not entirely eliminated. SARGANT (**15**) and others hold that monocotyledons are derived from dicotyledons. This occasional appearance of a multicellular archesporium may indicate that the dicotyledons from which these came

still had a multicellular archesporium, as the more primitive dicotyledons do yet.

Megaspores.—There are usually four megaspore nuclei formed. However, in a few instances, only one daughter cell divided. In many cases the wall separating these megaspore nuclei at the second division was seen to be forming (*figs. 14, 16, 19*) but always it had disappeared at maturity (*figs. 20, 23*). In a few cases there was no indication of a wall forming (*figs. 15, 17, 20*). This omission of all cell formation in the second division is the condition found in Cypripedium (**14**). The fact that Calopogon usually has the four megaspore nuclei was rather unexpected in this highly specialized group of the monocotyledons. For the tendency is not only to a row of three, due to the failure of one of the daughter nuclei to divide, but to the condition known as the mother cell functioning as a megaspore (COULTER and CHAMBERLAIN **7,** p. 80), which is common in monocotyledons. SCHNIEWIND-THIES (**16**) has shown that in Lilium the heterotypic division takes place within the embryo sac, only ephemeral walls being formed in either of the divisions from the mother cell to the megaspore. In Cypripedium it was found (**14**) that no wall appeared at the second division, and that two megaspore nuclei are used in forming the embryo sac. COULTER has shown (**6**) that this may be a very important point in connection with embryo sacs of more than eight nuclei. For if more than one megaspore nucleus enters into the organization of the sac, it is evident that more than eight nuclei in the sac would be the result of the usual five divisions from mother cell to egg found in angiosperms. So that while Cypripedium with its four-nucleate sac seems more reduced than Lilium, it really has just the same number of divisions from mother cell to egg (**14**).

BROWN (**4**) objects to calling these nuclei in Cypripedium megaspore nuclei, yet he proceeds to do it in Peperomia, although there is only the additional evidence of ephemeral walls. JOHNSON (**11**) has also shown these four nuclei in the tetrad position in Peperomia. The origin of the nuclei is identical in both Cypripedium and Peperomia, with the usual megaspore story except in the matter of wall—a mother cell passing from synapsis through the heterotypic and homotypic divisions. In Cypripedium a permanent wall appears at the heterotypic division, but none at all at the homotypic; in Peperomia

ephemeral walls appear at both divisions. This gives apparently four megaspore nuclei forming the sac in Peperomia, while only two enter into its organization in Cypripedium. It might be better to select another name for these nuclei, but it would always involve the explanation of their similarity to megaspore nuclei, with apparently no gain in clearness. For our notion of spores, extending back through pteridophytes and bryophytes, is that when a mother cell divides there are four spores produced, the spore being the cell formed by the heterotypic and homotypic divisions. The matter of walls would not seem to be so important as the behavior of the nuclear materials; for all sorts of variation in the kinds and shapes of these are found, these differences being related apparently to the environment of the spore. The egg is of course a descendant of only one megaspore, however many may enter into the construction of the sac. And it seems to involve less change from what have seemed to be the essentials to regard these as megaspore nuclei than to conceive of a mother cell functioning directly as a megaspore and yet proceeding to the very same heterotypic and homotypic divisions. This is more evident still when Calopogon is taken into consideration, as it seems to present a stage that might be considered intermediate between Cypripedium and the usual one. For here there are certainly two megaspore nuclei in the sac, one finally disintegrating, although some evidence of its division is present in two cases examined (*figs. 24, 25*).

This lack of walls is not very rare. LLOYD (**12**) reports them as usually absent in Rubiaceae, although the tetrad is formed. Here only one megaspore nucleus functions. The same condition is reported by CANNON (**5**) in Avena, and by SMITH (**17**) in Eichhornia. WIEGAND (**19**) finds the second divisions unaccompanied by walls in *Potamogeton foliosus*, and HOLFERTY (**10**) finds the wall omitted in the division of the micropylar daughter cell in *Potamogeton natans.* BILLINGS (**2**) reports one case of megaspores without separating walls in *Tillandsia usneoides.* And the many cases where the mother cell is said to function as a megaspore seem to represent the same condition, except that the non-functioning megaspores not only do not disintegrate but even divide and thus contribute to the contents of the embryo sac.

In Calopogon only one megaspore nucleus is used in forming

the sac, though two are certainly in it (*figs. 21, 22*), just as in Cypripedium. Both may divide, though no evidence beyond a late spirem or metaphase stage was seen, except in one case (*fig. 24*), and here the usual disintegration was taking place. So in this respect Calopogon might be regarded as suggesting what may have taken place in the ancestry of Cypripedium, as it passed from the usual tetrad or row of three to its present condition.

Fertilization.—So-called double fertilization is present here as in Cypripedium. It will be remembered that NAWASCHIN (13) reports it lacking in certain tropical orchids, while STRASBURGER (18) found it in orchids he investigated.

SUMMARY

1. There is usually one sporogenous cell which becomes the mother cell. But in many cases two mother cells are found, either contiguous or with nucellar tissue between.

2. Four megaspore nuclei are usually formed; occasionally the micropylar daughter cells in the second division either never appear or are ephemeral. This leaves two megaspore nuclei in the sac. But three of these megaspore nuclei disintegrate, the two from the micropylar daughter cell and the micropylar one in the sac.

3. The embryo sac is the usual eight-nucleate kind, developed in the usual way.

4. Pollinia are in massulae in four loculi. Tetrads are in any position, but there is no rounding-up of pollen grains. Tube and generative nuclei are formed before the pollen escapes. The pollen tube shows a tube nucleus and two male nuclei as it enters the ovary.

5. Double fertilization occurs.

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EXPLANATION OF PLATES VII–IX

All figures were drawn with a Bausch and Lomb camera. *Fig. 8* was made with Leitz no. 4 ocular and no. 7 objective, and *fig. 36* with no. 2 ocular and no. 7 objective; all others with Leitz no. 4 ocular and Bausch and Lomb $\frac{1}{12}$ objective.

The abbreviations used are as follows: *c* chalazal daughter cell and nucleus; *e* egg; *g* generative nucleus; *gc* generative cell; *m* micropylar daughter nucleus; *ms* megaspore nucleus; *p* polar nucleus; *s* synergid; *t* tube nucleus; ♂ male nucleus.

PLATE VII

FIG. 1.—Young ovule.
FIG. 2.—Young ovule with broader funiculus.
FIG. 3.—Archesporial cell differentiated; beginning of the integument.
FIG. 4.—Mother cell; synapsis almost complete.

FIG. 5.—Synaptic knot complete; integument much older.

FIG. 6.—Metaphase; thirteen chromosomes; multipolar spindle.

FIG. 7.—Spindle for division of mother cell.

FIG. 8.—Mother cell in same stages as *fig. 7*, but integuments much farther advanced.

FIG. 9.—Late anaphase; no evidence of wall formation.

FIG. 10.—Late telophase; wall separating the daughter nuclei.

FIG. 11.—Two daughter cells.

FIG. 12.—Two daughter cells, one of which is disintegrating.

FIG. 13.—Two daughter cells in mitosis.

FIG. 14.—Two daughter cells in telophase with walls forming; these are the megaspores.

FIG. 15.—Two daughter nuclei with no wall separating them; traces of the spindle still present.

FIG. 16.—Two daughter nuclei with no separating wall, but one of them larger than the other.

FIG. 17.—Chalazal daughter cell in telophase, with no trace of wall; micropylar daughter cell with chromosomes formed for division.

FIG. 18.—Chalazal daughter cell in telophase, with beginning of wall formation; micropylar daughter cell shows a slight thickening of the spirem.

PLATE VIII

FIG. 19.—Chalazal daughter cell in prophase; spirem short and thick; micropylar daughter cell in late telophase with wall forming.

FIG. 20.—Two megaspore nuclei of equal size; micropylar daughter cell undivided.

FIG. 21.—Embryo sac beginning to increase in size; one megaspore nucleus beginning to show evidence of mitosis; another megaspore nucleus disintegrating within the sac; the two megaspore nuclei from the micropylar daughter cell also disintegrating, with slight traces of the spindle still present.

FIG. 22.—About the same stage as *fig. 20*.

FIG. 23.—Four megaspore nuclei, one from each of the daughter cells disintegrating with its own cytoplasm, the other one in each case in good condition for development, although the chalazal nucleus is the larger.

FIG. 24.—Micropylar daughter cell with chromosomes formed for mitosis; one megaspore nucleus in sac developing, the other already divided, and both nuclei disintegrating.

FIG. 25.—Chalazal megaspore nucleus with spindle; the other megaspore nucleus in the sac in late spirem, or metaphase; the micropylar daughter cell nucleus in spirem stage.

FIG. 26.—Two sporogenous cells in one ovule.

FIG. 27.—Two mother cells in one ovule.

FIG. 28.—Two mother cells with nucellar tissue between them.

FIG. 29.—Two mother cells.

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PAGE ON GALOPOGON

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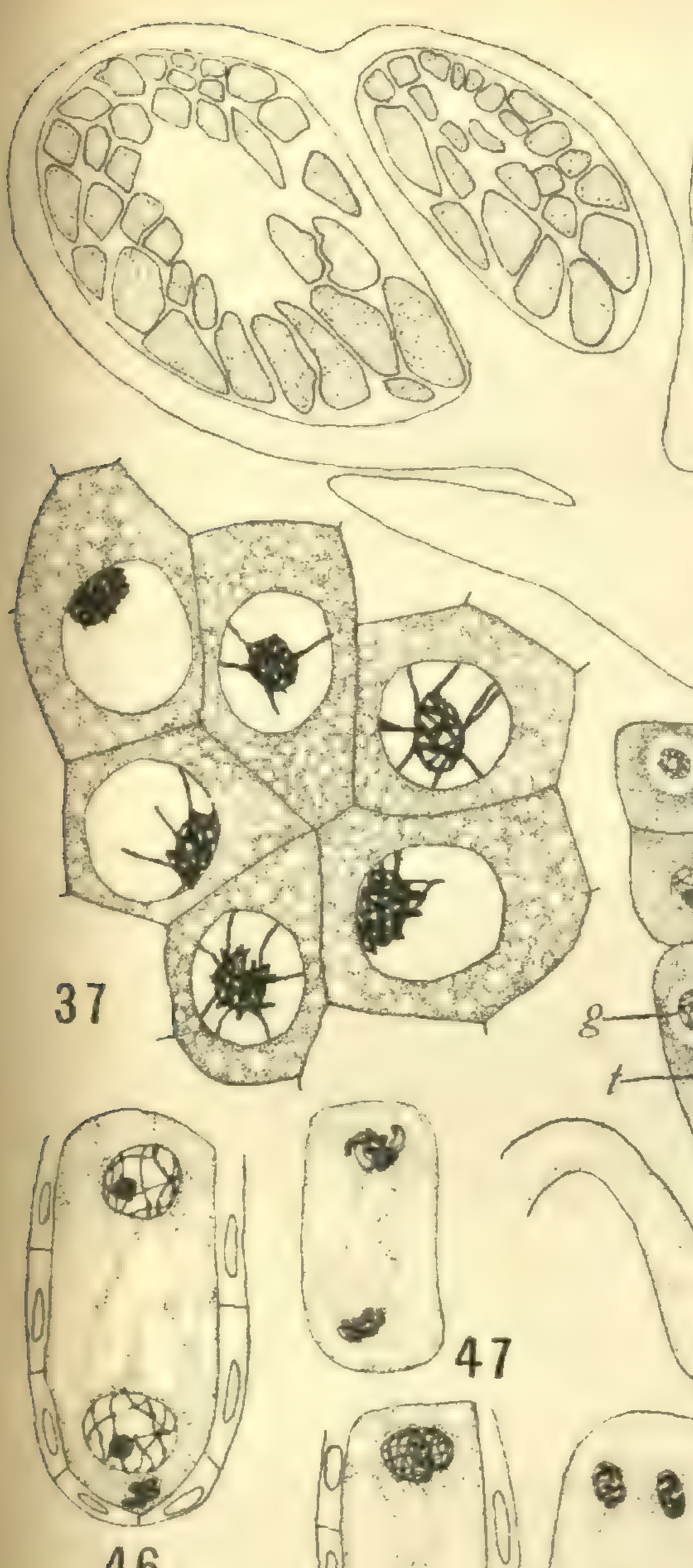

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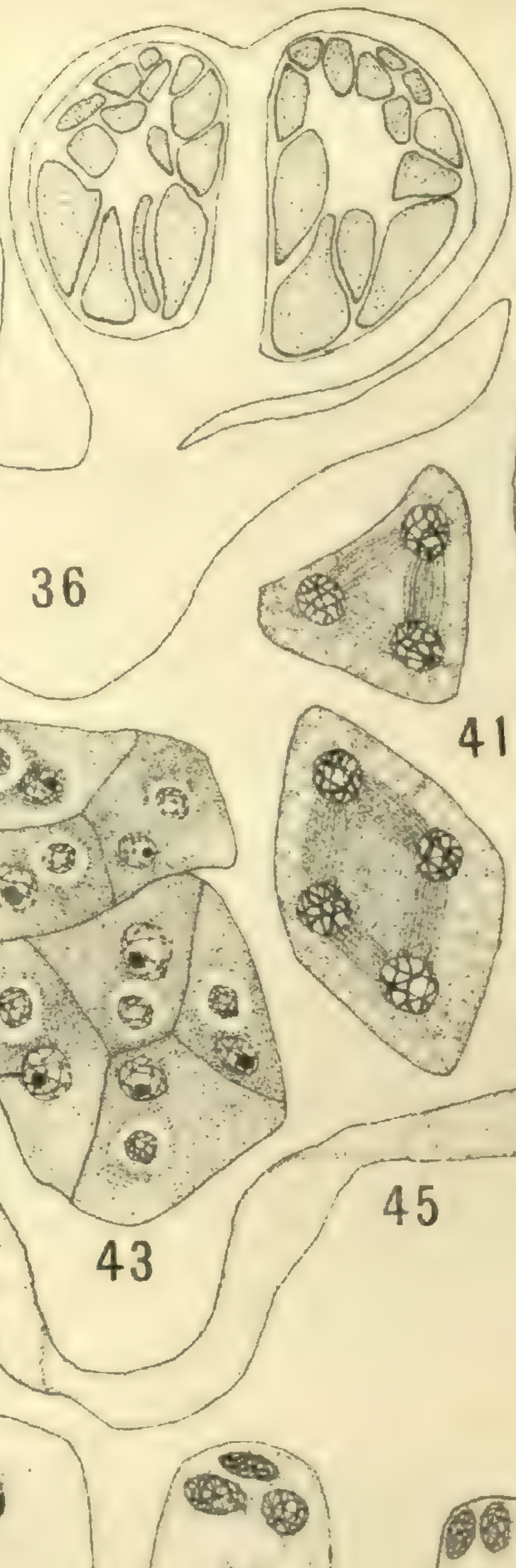

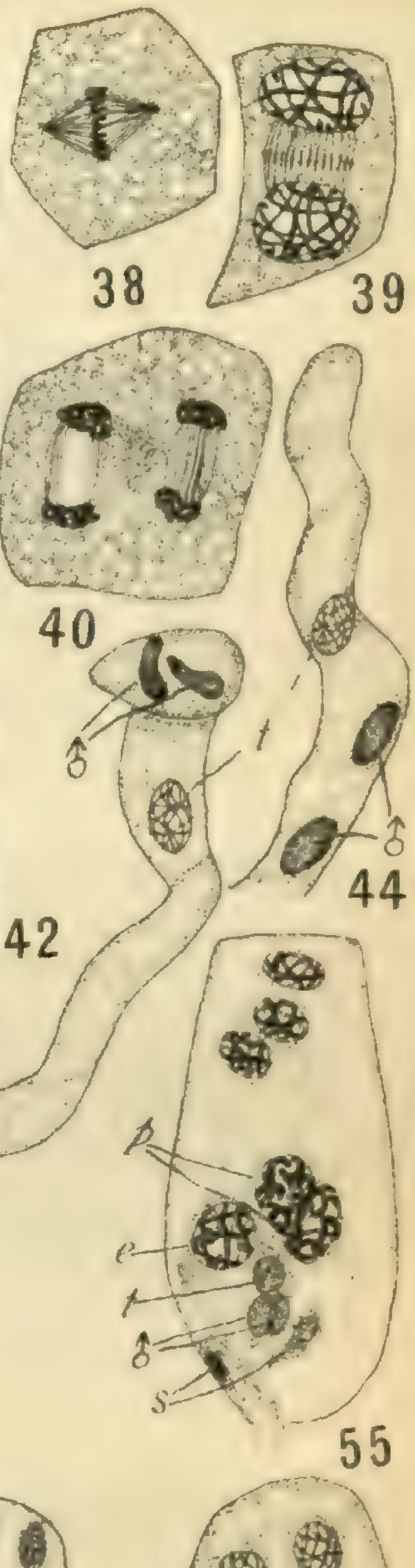

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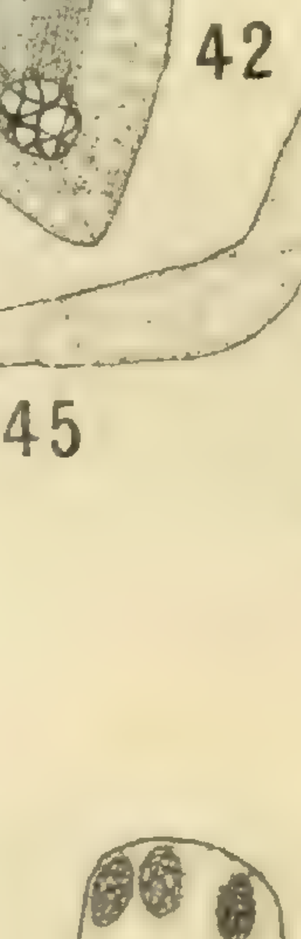

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PAGE ON CALOPOGON

FIG. 30.—Two mother cells after recovery from synapsis.

FIG. 31.—Two mother cells with several cells of nucellar tissue between; ovule cut across.

FIG. 32.—Ovule with two daughter cells; and probably another mother cell beside it, or another daughter cell with slight traces of the second disintegrating.

FIG. 33.—Eight megaspores from two mother cells.

FIG. 34.—Two-celled embryo sac, three disintegrating megaspore nuclei, and a mother cell recovered from synapsis.

FIG. 35.—One mother cell in metaphase, the other already divided, giving two daughter nuclei without separating wall.

PLATE IX

FIG. 36.—Diagram of anther, showing four pollen masses; massulae shaded.

FIG. 37.—Pollen mother cells in synapsis.

FIG. 38.—Pollen mother cell with spindle.

FIG. 39.—Pollen mother cell in telophase.

FIG. 40.—Telophase of second division.

FIGS. 41, 42.—Pollen tetrad; microspore nuclei.

FIG. 43.—Two tetrads, each pollen grain of which shows tube and generative nucleus.

FIG. 44.—Pollen tube from within ovary.

FIG. 45.—Pollen tube near ovule.

FIG. 46.—Two-celled embryo sac.

FIG. 47.—Both nuclei in sac in mitosis.

FIG. 48.—Four-celled embryo sac: three disintegrating megaspores.

FIG. 49.—Four-celled sac; traces of spindle of preceding division; megaspore nucleus within sac disintegrating.

FIG. 50.—Four-celled sac; prophase for next division; traces of disintegrating megaspore nucleus and micropylar daughter cell.

FIG. 51.—Eight-celled sac; traces of spindles of preceding division.

FIG. 52.—Nuclei beginning to assume usual position in sac.

FIG. 53.—Sac ready for fertilization; egg apparatus of egg and two synergids; two polars, one a sister to the egg; three antipodals; pollen tube between integuments.

FIG. 54.—Pollen tube within the sac; egg and two polars in late spirem or metaphase.

FIG. 55.—Pollen tube in sac; egg with thick spirem; polars with chromosomes.

FIG. 56.—Double fertilization.

ON MESARCH STRUCTURE IN LYCOPODIUM[1]

EDMUND W. SINNOTT

(WITH PLATE X)

Halftone

The position of the protoxylem in relation to the later-formed elements of the wood has been the subject of careful investigation in the various groups of vascular plants, both fossil and living, and has often been brought forward as evidence of the relationship of one group to another.

In his description of the anatomy of a specimen of Sigillaria, in 1839, BRONGNIART (**1**) showed the central cylinder to be formed of a ring of vascular bundles, each composed of an inner, primary part, and an outer, secondary part, the latter characterized by the radial arrangement of its elements. The small spiral elements lie on the outer edge of the primary wood, which was all developed in a centripetal direction, while the secondary wood grew centrifugally. The protoxylem is thus surrounded by later-formed elements.

In his classic work on the anatomy of the cycads, METTENIUS (**2**), in 1861, described the structure of the vascular bundle in the petiole and blade of certain living cycads. He showed that in the leaf-trace, as it leaves the cylinder of the stem, the protoxylem is all on the inside of the later-formed, mostly secondary, wood, but that as the bundle ascends into the petiole, the later-formed wood gradually bends around inwardly and incloses the spiral elements. Finally the centrifugal wood, which is probably all secondary, is greatly reduced, and separated from the protoxylem by parenchyma, while the centripetal wood forms the bulk of the bundle, and bears at its outermost point the cluster of spiral tracheids. METTENIUS (**2**, p. 582) noticed the resemblance between this bundle and one of the stem bundles of Sigillaria, and remarks that the much greater development of centrifugal secondary wood in the latter is due to the fact that it belongs to the vascular system of the stem.

RENAULT (**3**), however, was the first to really suggest the affinity

[1] Contributions from the Phanerogamic Laboratories of Harvard University, no. 16.

of the cycads with the Sigillariae on the basis of a common possession of this character. He carefully distinguished the irregularly arranged centripetal wood from the radially disposed centrifugal wood of the sigillarian bundle, and was the first to describe the structure of the leaf-trace in this genus. The elements in the leaf are also formed in two directions, those on the outside often being radially arranged. This structure making the comparison with the cycads still closer, RENAULT comes to the conclusion (**3,** p. 281) that "les Cycadées actuelles qui possèdent dans la structure du cordon foliaire cette analogie si frappante avec certaines plantes houillères, n'en sont que les représentants amoindris et en voie de décadence."

RENAULT has applied the term diploxylic to bundles possessing centrifugal as well as centripetal wood, and has grouped together Sigillaria and Poroxylon under the head DIPLOXYLÉES. Count SOLMS-LAUBACH (**4,** p. 263), however, called attention to the fact that where all the centrifugal wood is secondary, the original primary bundle, which thus has its oldest elements on the very outside, is of a different type from one where there is primary wood on the outside, as well as on the inside, of the protoxylem. For the former type of primary bundle he suggested the name exarch, and for the latter type mesarch, terms which have since been in current use with those meanings. RENAULT'S diploxylic implies the presence of secondary wood.

On the characteristic possession of one of these two main types of primary bundle, modern writers have often divided living and fossil vascular cryptogams into two great groups—the Fern series generally, as typically mesarch, and the Lycopodiales, fossil and living, as typically exarch.

This general rule, however, does not hold at all closely. Among living ferns, there are numerous instances of exarch structure, for example in the stem of *Trichomanes scandens*, of *Lygodium dichotomum*, and of *Loxoma Cunninghamii.* The same holds true of fossil ferns and their allies. Zygopteris shows both internal and external protoxylem, while Megaloxylon is clearly exarch.

Among the typically exarch forms, on the other hand, RENAULT (**3**) has figured mesarch leaf-traces in the Sigillariae. They are also found in certain species of Lepidodendron. Numerous cases of such development also occur in the modern relatives of this group. In

the center of each stem bundle of Tmesipteris there is a cluster of initial elements, the breaking-down of which often results in the formation of a lacuna. Although the cylinder of the stem of Selaginella is characteristically exarch, GIBSON (**5**) has noted, in the case of the protostelic *S. spinosa*, that in the trailing portion of the stem, all the protoxylem is in the center of the cylinder, completely inclosed by metaxylem. The root in the whole genus is also distinctly mesarch. In Phylloglossum, it is well known that the tubular stele at the base of the peduncle, the ring of bundles into which this divides higher up, and the traces of the sporophylls, have their first-formed wood elements surrounded on all sides by later ones. Exarch development seems entirely lacking, both in this genus and in Tmesipteris.

In Lycopodium, however, precisely the reverse seems at first sight to be true, and I have been unable to find a recorded instance of the occurrence of mesarch development in the genus.

In an endeavor to determine the presence or absence of such a character, the following species and varieties of Lycopodium were investigated: *L. inundatum* L., and var. *Bigelovii* Tuckerm.; *L. lucidulum* Michx.; *L. annotinum* L.; *L. obscurum* L.; *L. tristachyum* Pursh; *L. complanatum* L. var. *flabelliforme* Fernald; and *L. clavatum* L.

L. inundatum and its variety are somewhat delicate forms, and have by far the most poorly developed vascular system of the species looked at. In both, the xylem rays are few in number (3–6), and along the much broadened end of each extends a row of crushed protoxylem elements. There is no indication of metaxylem outside of these. In the leaf-trace, however, especially at a little distance from the cylinder, the smaller elements, ringed or loosely spiral, tend to become clustered at the center of the bundle. Just before the trace enters the leaf, these elements break down, leaving a protoxylem lacuna, completely surrounded by later-formed, closely spiral elements. This is perfectly evident in both transverse and radial sections (*figs. 1, 2*).

In the case of *L. lucidulum*, the central cylinder is also small, in comparison to the diameter of the stem, and its xylem rays are few in number and broadened at the ends. As in *L. inundatum*, however, there is no indication of centrifugal wood in the cylinder itself, but the

leaf-traces, though composed of a comparatively small number of cells, show in nearly every case a mesarch structure (*fig. 3*).

The remaining species studied have much better developed vascular tissues. Their cylinders are in general very similar, being composed of a much larger number of xylem rays, which end more or less acutely. In all the species, metaxylem cells occur just outside the protoxylem of the cylinder. In *L. tristachyum* and *L. complanatum* var. *flabelliforme*, this mesarch development is not common, but in *L. obscurum* and *L. clavatum*, especially the latter, it is very noticeable. In vertical section, the centrifugal elements are seen to be reticulate, not scalariform, as in the rest of the metaxylem (*figs. 4, 6*). The exceptional development of this structure in *L. clavatum* may be due to the fact that its vegetative growth is more luxuriant than that of any of the other species studied. In all these forms, the leaf-trace again is very clearly mesarch (*figs. 7–9*). This is especially conspicuous, perhaps, in *L. tristachyum* and *L. complanatum* var. *flabelliforme*, where a large protoxylem lacuna occurs in the center of the trace (*fig. 9*).

In all the species, the leaf-trace, as it leaves the cylinder, is very small, but rapidly increases in size in the cortex, where it possibly serves to store water.

The development of the protoxylem of the central cylinder is always from without inward. The single row of centrifugal metaxylem elements occurs directly outside the earliest-formed xylem cells. Sections through the growing-point of *L. clavatum* showed that the metaxylem elements on both sides of the protoxylem developed nearly simultaneously. In the leaf-trace, also, as was shown by a very young stem of *L. complanatum* var. *flabelliforme*, the elements surrounding the protoxylem are all developed at the same time.

The leaf-trace of *Selaginella rupestris* (L.) Spring was investigated, and though composed of a very small number of cells, it showed a strong tendency toward mesarch arrangement. It is interesting to note here an observation of GIBSON (**6,** p. 151) on the leaf bundle of Selaginella. "In the upper third of the leaf the protoxylem elements become accompanied by several short reticulate tracheides which flank the spiral tracheides, and in section (e. g., of *S. Braunii*) may inclose the spiral elements completely."

It is noteworthy that in certain of the fossil Lycopodiales, as above mentioned, there is clear evidence of the possession of mesarch structure in the primary wood of the leaf-trace.

That there is no sharp line of cleavage between mesarch and exarch vascular cryptogams is thus perfectly clear. Among the main groups, we have numerous instances of both types of development, and the one merges, little by little, into the other. In Lycopodium, the centrifugal wood of the stem, when present, consists of only a few tracheids; in such forms as Lyginodendron, for instance, and in many ferns, the spiral elements are near the outside, while in Tmesipteris and Phylloglossum, they are in the very center. The variability of the position of the protoxylem in the primary wood bundle, and its consequent unreliability as a phylogenetic character, cannot be too strongly emphasized.

While the structure of the primary bundles of the vascular cryptogams cannot be used as a guide to their interrelationships, it furnishes an excellent character for the whole group, both living and fossil forms. The presence in the stem of *centripetal primary wood, continuous with the protoxylem*, is the distinguishing mark of these plants. The older French botanists appear to have been perfectly right in characterizing their *bois centripète* as cryptogamic wood, for the Sphenophyllales, Lycopodiales, and Filicales show this structure very clearly. The only apparent exception[2] to the rule is the calamitean series, including the modern Equisetales. A species of Calamites, however, the so-called *C. pettycurensis*, or Protocalamites, has recently been shown to have well-developed centripetal wood on the inner face of the protoxylem canals of the stem (SCOTT **7**). Further, EAMES (**10**), working in this laboratory, has distinguished a mesarch condition, with consequent presence of centripetal xylem, in the traces of the vegetative and reproductive leaves of several living species of Equisetum. It thus seems certain that centripetal wood was once well developed in the Calamites and their allies, but in the process of time has been almost entirely lost.

It is worthy of note that though secondary wood is often present in the vascular cryptogams, and may constitute the bulk of the central cylinder, the protoxylem is intimately associated with the centrip-

[2] Exclusive of that aberrant group of ferns, the Ophioglossales.

etal primary wood. In the higher plants, the centrifugal xylem preponderates, and the protoxylem becomes continuous with it, even in the cases where centripetal elements are present. The only exceptions to this rule occur in foliar bundles. The leaves of living cycads and of the Cordiates show true cryptogamic wood in their vascular strands, with the protoxylem closely associated with it, though in all cases centrifugal secondary wood is present. This persistence of a cryptogamic structure in the leaf, among the higher plants, furnishes good evidence of the conservatism of the foliar bundle. Scattered centripetal elements appear in the peduncle of certain cycads and in the cotyledons of Ginkgo, also seats of vestigial characters, and centripetal wood is fairly well developed in the leaf of Prepinus (JEFFREY **9**) and in the stems of many of the Cycadofilices; but in all these cases, the bundle is of the higher type, the protoxylem being continuous with the *centrifugal* wood.

The structure of the vegetative stem, of the floral axis, and of the leaf, in certain living Cycadaceae, shows clearly the transition from the lower type of wood to the higher. In the stem, the protoxylem joins the centrifugal wood and there are no centripetal tracheids. In the stalk of the male cone of *Stangeria paradoxa*, and to a less extent in the male and female peduncles of three other species of cycads, as shown by SCOTT (**8**), scattering centripetal xylem elements are present. They are not connected with the protoxylem, however, but are separated by parenchyma from the rest of the bundle, which otherwise is exactly similar to those found in the stem. In the petiole and blade of the cycad leaf, however, there is more than this faint suggestion of ancestral structures; for here, as above described, true cryptogamic wood occurs, consisting of well-developed centripetal elements directly continuous with the protoxylem. In the petiole, and sometimes in the blade, centrifugal wood is present as well. This is separated by parenchyma from the protoxylem, and appears to be largely, if not entirely, laid down by cambial activity. Its resemblance to a sigillarian bundle, as noted by RENAULT, is thus pretty exact. SCOTT, however, believes that some, at least, of the centrifugal wood is primary, and consequently that true mesarch development takes place, a condition which he compares to that found in the stem bundles of *Lyginodendron Oldhamium*. He presents the mesarch structure

of the peduncle and of the leaf as evidence of the affinity of modern cycads to the fossil Lyginodendreae and Poroxyleae. While the conclusion as to affinities is very probably correct, the argument is not entirely convincing. The cryptogamic ancestry of the cycads is proven, but that is all; for we have already seen that the possession of mesarch primary wood is limited to no one group of plants, and that its presence or absence is of no value as phylogenetic evidence.

CONCLUSIONS

The position of the protoxylem in the primary wood bundle of vascular cryptogams is very variable, for in all of the groups it may be almost anywhere in the strand. It is consequently of little value in determining the relationship of any group to other groups or to the higher plants. A constant primitive feature in the vascular cryptogams, however, is the presence in the stem of cryptogamic wood—centripetal xylem continuous with the protoxylem elements. Whenever such a feature occurs in any of the higher plants, it can be used as evidence in tracing their affinity only with vascular cryptogams in general, not with any particular group of them.

I desire to express my thanks to Dr. M. A. CHRYSLER and to Mr. A. J. EAMES for assistance in procuring material, and especially to Professor E. C. JEFFREY for advice during the course of the work.

This investigation was carried on in the Phanerogamic Laboratories of Harvard University.

HYANNIS, MASS.

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EXPLANATION OF PLATE X

Fig. 1.—*Lycopodium inundatum* var. *Bigelovii;* longitudinal radial section through a leaf-trace. ×500.

Fig. 2.—*Lycopodium inundatum* var. *Bigelovii;* transverse section of a leaf-trace near the margin of a mucilage cavity. ×500.

Fig. 3.—*Lycopodium lucidulum;* transverse section of a leaf-trace. ×500.

Fig. 4.—*Lycopodium clavatum*, longitudinal radial section through the margin of the central cylinder, showing the single centrifugal metaxylem element on the right. ×500.

Fig. 5.—*Lycopodium clavatum;* transverse section through the margin of the central cylinder at the point of departure of a leaf-trace; the dark line of crushed cells marks the position of the protoxylem. ×500.

Fig. 6.—*Lycopodium obscurum;* longitudinal radial section through the margin of the central cylinder, showing centrifugal metaxylem as in *fig. 4.* ×500.

Fig. 7.—*Lycopodium clavatum;* longitudinal radial section through a leaf-trace. ×500.

Fig. 8.—*Lycopodium clavatum;* transverse section of a leaf-trace. ×500.

Fig. 9.—*Lycopodium tristachyum*; transverse section of a leaf-trace. ×500.

BRIEFER ARTICLES

SOME HITHERTO UNDESCRIBED PLANTS FROM OREGON

Mr. WILLIAM C. CUSICK has submitted to the writer for determination an interesting series of castillejas and senecios, collected by him during the past few years in the Wallowa Mountains of northeastern Oregon. The material is copious and for the most part falls readily into well-known species. There are, however, a few plants which do not satisfactorily accord with any described species; these may be here characterized as follows:

Castilleja chrysantha, n. sp.—Herbacea perennis tota planta glanduloso-villosa: caulibus erectis vel ascendentibus 0.5–3^{dm} altis: foliis lineari-lanceolatis vel lanceolatis vel interdum subovatis 1–3^{cm} longis 1.5–10^{mm} latis integris vel trifidis trinerviis crispo-hirsuto-glandulosis, laciniis linearibus acutis patentibus; inflorescentiis spicatis 1.5 to 14^{dm} longis; bracteis anguste lanceolatis vel lanceolatis integris vel trifidis flavo-viridibus vel non rarius paululo purpurascentibus; calyce 13–18^{mm} longo antice et postice subaequaliter fisso ad circiter medio altitudinem connato, laciniis oblongis et integris ad apicem rotundatis vel bilobatis, lobis oblongis obtusis exteriore glanduloso-villosis viridibus vel aureoviridibus vel rarius paululo purpurascentibus; corolla 16–20^{mm} longa flava; galea recta 4–6^{mm} longa; labium 2.5–3^{mm} longum, labii lobis oblongo-ovatis obtusis calycem superantibus; capsula oblongo-elliptica circiter 8^{mm} longa breviter acuminata glabra.

In wet meadows at the head of West Eagle Creek, Wallowa Mountains, Oregon, altitude 2135^{m}, 15 August, 1907, *William C. Cusick*, no. 3200^{b} (type hb. Field Mus. Cat. no. 225021); in moist soil near the source of the Imnaha, Wallowa Mountains, altitude 2440^{m}, 14 August, 1906, *William C. Cusick*, no. 3124 (hb. Field Mus.); on dry mountain sides, Kettle Creek, Oregon, altitude 1830^{m}, July, 1906, *William C. Cusick*, no. 3103^{a} (hb. Field Mus.); in wet meadows, Wallowa Mountains, Oregon, altitude 2135^{m}, 19 August, 1907, *William C. Cusick*, no. 3205^{a} (hb. Field Mus.); source of the Wallowa River, altitude 2440^{m}, 15 August, 1908, *William C. Cusick*, no. 3324^{c} (hb. Field Mus.); wet subalpine prairie of the Wallowa Mountains, 28 August, 1898, *William C. Cusick*, no. 2110 (hb. Field Mus.), distributed as "*Castilleia oreopola* Greenman, white-flowered form."

The habit and glandular pubescence of this species suggests a close relationship to *C. viscidula* Gray and *C. Covilleana* Henderson. From the former *C. chrysantha* differs in having a longer calyx with oblong and rounded or obtuse

instead of acute ultimate calyx divisions; the lip is longer in proportion to the galea, and the pubescence is more villous. From *C. Covilleana* it differs in the less deeply cleft bracts, in the oblong blunt divisions of the calyx, and in the longer exserted lip of the corolla.

Castilleja fraterna, n. sp.—Herbacea perennis; caulibus caespitosis numerosis ascendentibus plus minusve flexuosis 0.5–1.5^{dm} altis viridibus vel purpurascentibus in partibus inferioribus glabris, superioribus villosis hinc inde glanduloso-pubescentibus; foliis linearibus vel lanceolatis 1–2.5^{cm} longis integris vel trifidis pubescentibus, laciniis linearibus acutis patentibus; spicis pauci-multifloris 5^{cm} vel minus longis; bracteis plerumque trifidis rubris; calyce circiter 2^{cm} longo exteriore pubescente antice quam postice fissiore laciniis lateralibus 2-lobatis, lobis lanceolato-oblongis obtusis rubris; corollis 2.5–3^{cm} longis exteriore plus minusve pilosis flavis et rubellis; galea erecta circiter triplo brevior quam tubus flava vel flavo-viridia, margine rubello, labio 2.5–3.5^{mm} longo basi triplicato plerumque atro-viridi, labii lobis anguste-ovatis acutis vel obtusis ad apicem rubicundis; capsula oblonga 8–10^{mm} longa brevi-acuminata acuta glabra.

In alpine regions of the Wallowa Mountains, Oregon, 14 August, 1906, *William C. Cusick*, no. 3125 (type, hb. Field Mus. Cat. no. 225016); and in the same locality, 27 August, 1907, *William C. Cusick*, no. 3222° (hb. Field Mus.).

Mr. CUSICK states that the plants here cited were associated with Salix, and that they grew especially in thickets of *S. sitchensis*. The numerous subflexous stems, ascending from a common base, each terminated by a conspicuous inflorescence variegated with green, red, and yellow, render it an extremely attractive species.

Castilleja oresbia, n. sp.—Herbacea perennis basi lignosa; caulibus simplicibus erectis vel ascendentibus 1.5–2^{dm} altis crispo-hirsutis; foliis lineari-lanceolatis et integris vel trifidis 1–4^{cm} longis 2–5^{mm} latis crispo-pubescentibus, laciniis linearibus acutis patentibus; inflorescentiis terminalibus dense spicatis 1.5–4.5^{cm} longis; bracteis saepissime trifidis, lobis lateralibus linearibus obtusiusculis, lobo intermedio majore lanceolato-ovato; calyce 10–14 ^{mm} longo exteriore pubescente antice et postice aequaliter fisso, lobis oblongis bidentatis; corolla flava 14–17^{mm} longa glabra, galea recta circiter 6^{mm} longa, labii lobis lineari-elongatis 4^{mm} longis acutis; capsula oblonga acuta circiter 1^{cm} longa glabra.

On dry mountain sides, Kettle Creek, Oregon, altitude 1830^{m}, 19 August, 1907, *William C. Cusick*, no. 3201^{a} (hb. Field Mus. Cat. no. 225022).

In habit and general appearance this species resembles most closely *C. rustica* Piper, from which it differs in having shorter, more densely flowered, and less villous spikes, and in the characters of the corolla. A marked distinguishing

feature of *C. oresbia* is the straight corolla with the conspicuously long lobes of the lower lip.

SENECIO HOWELLII Greene, var. **lithophilus,** n. var.—A forma typica recedit foliis multo amplioribus, maximis 3–15^{cm} longis 1–3.5^{cm} latis integris vel inaequaliter et alte dentatis.

In stony dry soil, Imnaha, Wallowa Mountains, Oregon, about 1830^{m}, 12 August, 1906, *William C. Cusick*, no. 3129 (hb. Field Mus.).

Mr. CUSICK'S specimens under the number here cited seem at first glance to be more than of varietal significance, but a comparison with the type of *S. Howellii* and a considerable suite of specimens from the Gray Herbarium and in the herbarium of the Field Museum show the plant in hand to be only an extreme variation with large, broad, entire or coarsely and unequally dentate leaves.—J. M. GREENMAN, *Field Museum of Natural History, Chicago.*

CURRENT LITERATURE

BOOK REVIEWS

Ecology of plants

For a dozen years English and American botanists have been more or less hopefully awaiting a translation of WARMING'S *Plantesamfund*, which was promptly translated into German and thus made available to a larger audience. A peculiar accumulation of misfortunes of one kind or another prevented an English edition of this epoch-making work, but at last WARMING'S contributions are made available to all English and American botanists, and in the most happy way possible, through the preparation of an essentially new book.[1] The author has excellent command of the English language, having frequently contributed articles in this tongue. The new volume was written in English by the author himself, assisted by Dr. MARTIN VAHL, and was prepared for publication by Drs. PERCY GROOM and I. B. BALFOUR. A preface written by the author calls attention to the more fundamental changes in the English work; these are so many and so important that an extended review is necessary.

The introduction contains considerable new matter concerning growth forms (*Lebensformen* or *Vegetationsformen*), together with a new and rather satisfactory classification of them. There are six categories of growth forms: heterotrophic, aquatic, muscoid, lichenoid, lianoid, and all other autonomous land forms. The final category is, of course, much the largest, and is subdivided into monocarpic and polycarpic forms. Monocarpic growth forms may be aestival annuals, hibernal annuals, or biennials to perennials. The polycarpic forms are much more numerous, and their subdivision is based largely on the idea of the character of their protection during the severest seasons, taking account of such things as the duration of the vegetative shoot, the length and direction of the internodes, the position of the renewal buds, the bud structure, size, leaf duration, and the adaptation of the nutritive shoot to transpiration. The sub-classes of polycarpic forms are renascent plants (subdivided into plants with multicipital rhizomes, mat geophytes, and traveling or rhizome geophytes), rosette plants (subdivided into ordinary rosettes and tree rosettes), creeping plants, and plants with erect long-lived shoots (subdivided into cushion plants, undershrubs, soft-stemmed plants, succulent-stemmed plants, and woody plants with long-lived lignified stems).

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[1] WARMING, EUG., assisted by MARTIN VAHL, Oecology of plants; an introduction to the study of plant communities. Prepared for publication in English by PERCY GROOM and ISAAC BAYLEY BALFOUR. Royal 8vo. pp. xii+424. Oxford: Clarendon Press. 1909. Cloth, $2.90; morocco, $3.25.

The first and second sections, dealing with ecological factors and their action and the communal life of organisms, are but little changed, except that they are brought up to date, as is true of every part of the book to a most remarkable degree. Sun and shade plants are called heliophytes and sciophytes respectively, and their leaves are denominated heliophylls and sciophylls. The third section brings together the parts referring to the adaptations of land and water plants, distributed under various heads in the Danish and German editions. Much more vital, however, involving new conceptions rather than rearrangement of material, is the new classification of plant formations. Here the author departs widely from his former fourfold classification of plants into hydrophytes, mesophytes, xerophytes, and halophytes. The new conception is that there is a fundamental twofold subdivision into land plants and water plants. The land plants are subdivided further into twelve primary groups, thus making with the hydrophytes thirteen main classes of plant formations, in place of the four classes of previous editions. For the new classification, the author states that Dr. VAHL is largely responsible, and especially for those following the psammophytes, as noted below.

In former editions it will be recalled that WARMING objected, and with good reason, to the word formation as an ecological unit, largely because of its varied use by different authors. But language is a peculiar thing, and ill-chosen words often stick. It has been so with formation, and the author now attempts to delimit the word, regarding a formation as "a community of species, all belonging to definite growth forms, which have become associated together by definite external characters of the habitat to which they are adapted." The chief classes of formations are microphyte, moss, herb, undershrub, shrub, and forest, and individual formations may be simple, compound, or mixed. An association is defined as "a community of definite floristic composition within a formation, a floristic species of a formation which is an oecological genus." The conception of a formation as an ecological genus and an association as an ecological species is now becoming generally accepted in principle, but this concrete statement by the father of modern ecology should make its acceptance universal.

The new classification of formations, with some of the leading sub-classes under each, follows.

A. Soil wet; water available. (1) Hydrophytes; subdivided into plankton (further split up into haloplankton, limnoplankton, and saproplankton), cryoplankton (including the microphytes of ice and snow), hydrocharid formations or pleuston, the lithophilous benthos (in place of the nereids), the benthos of loose soil (including hot spring microphytes, sand algae, saprophytic microphytes, and the enhalid and limnaea formations, as in previous editions). (2) Helophytes or swamp plants; subdivided into reed swamps and bush swamps.

B. Soil physiologically dry. (3) Oxylophytes or plants of sour (i. e., acid) soil, characterized largely by xeromorphy; subdivided into low moors, grass heaths, high moors, moss and lichen heaths (or tundra), dwarf shrub heaths, and bushland or forest on acid soil. (4) Psychrophytes or plants of cold soil,

including chiefly the subglacial fell-fields. (5) Halophytes; subdivided into lithophilous, psammophilous, and pelophilous halophytes, salt swamps and deserts, and littoral swamp forests (mangrove swamps).

C. Soil physically dry and dominant in determining the vegetation. (6) Lithophytes; subdivided into the true lithophytes (chiefly lichens) chasmophytes, and shingle and rubble plants. (7) Psammophytes or sand plants. (8) Chersophytes or waste plants (i. e., ruderals); subdivided into waste herbage and bushland on dry soil.

D. Climate dry and dominant in determining the vegetation. (9) Eremophytes, or plants of steppes and deserts; subdivided into deserts, shrub steppes, and grass steppes (including prairies). (10) Psilophytes or savanna plants; subdivided into thorny savanna, true savanna, and savanna forest. (11) Sclerophyllous plants; subdivided into garique, maqui, and sclerophyllous forest.

E. Soil physically or physiologically dry. (12) Conifers.

F. Soil and climate favorable to mesophilous formations. (13) Mesophytes; subdivided into arctic and alpine mat grassland, meadow, pasture, mesophytic bushland, deciduous dicotylous forests, and evergreen dicotylous forests.

The classification here outlined does not strike the reviewer as an improvement over the one abandoned. For the most part the new groups of land formations are segregates from the old term xerophyte. It is true that the latter term had become unwieldy, but it is questionable whether the difficulties are solved. Among the advantages of the new scheme is the recognition of close relationship between heath and moor plants, which together form the category oxylophytes, and also the close ecological relationship between these plants and the plants of cold and salty soils, as emphasized by SCHIMPER. It is gainful, too, to put the edaphic xerophytes (lithophytes, psammophytes, chersophytes) into one category and climatic xerophytes (eremophytes, psilophytes, sclerophylls) into another. Among the disadvantages of the new arrangement are many instances where unrelated things are placed together and related things are separated. The most conspicuous case of the former is seen in class 12, the conifers. While this group is a floristic unit, and even an ecological unit from the anatomical standpoint, it is far from being a geographic unit of any sort. It would seem better to put many conifers with the lithophytes and psammophytes, while others are certainly oxylophytes, and others still pronounced mesophytes, the most mesophytic forests of the United States being dominated by conifers. Again, classing the plants of steppes and deserts together as eremophytes does an injustice to the American prairie, much of which is essentially mesophytic. Repetitions in listing the plant formations of a region would be certain to occur, for example as between lithophytes and psammophytes, oxylophytes and psychrophytes in northern regions, etc. It also seems unfortunate that so many Greek derivatives are employed. Nowadays we are getting more and more to abandon the classics and their lengthy derivatives for the vigorous and familiar terms of the vernacular, and it seems a pity to have to brush the dust once more from our Greek lexicons. The antiquated spelling of ecology (oecology) is unfortunate, and is

made more striking by contrast with the frequently used economy, which has the same reason for appearing as oeconomy.

While words of adverse criticism seem necessary here and there, one may write volumes of praise. WARMING'S *Plantesamfund* will be for all time the great ecological classic, and the English volume now before us is the most important ecological work in any language. It is at the same time an old book and a new, a translation of the masterpiece of 1895 and a compendium of the ecological thought of 1909. WARMING has been contributing to ecology for more than forty years, and is the undisputed Nestor of the subject, but unlike many a Nestor, WARMING incarnates the ambitions and plasticity of youth. It will be pleasing to American ecologists to see the remarkable recognition accorded to their work in this new volume. The German edition of 1896 contained but one American title, though a half-dozen more might have been included. Now there are 600 titles in all, almost exactly twice the number published in 1896, and it is not unfair to say that half of the ecological work thus far accomplished is represented by the added titles of the last thirteen years. It will be flattering to Americans to note that 115 of the 300 new titles represent American contributions, a record that measures up well with the bare half-dozen that might have been named in the original 300. The new edition has ample footnote references, adding inestimably to the service of the work. The absence of illustrations will be a source of disappointment to many, but it accounts in large part for the extremely low price of the volume, a price that will insure a sale that has been accorded to no ecological work in the English language.—HENRY C. COWLES.

Experimental morphology

Although experimental morphology received its original impetus from observations on plants, during the last few years there has been a great dearth of botanical contributions to this field as compared with what the zoologists have done. It is partly with the hope of stimulating anew botanical exploration in this fascinating field that GOEBEL has published a course of lectures which he delivered in 1906–1907.[2] The field of experimental morphology, perhaps from necessity, is arbitrarily limited to what is often called developmental physiology, no reference being made to the direct influence of gravity, light, etc., on form. The author refers those who wish information on such topics to PFEFFER'S *Physiology* and his own *Organography*. Furthermore, the material presented is mainly from the higher plants, reference being made to KLEBS for similar material on the lower forms.

Especially interesting is the first chapter, which considers the mission of experimental morphology, for here the author reveals his philosophy. It is clear that GOEBEL goes about as far as KLEBS in referring plant phenomena to the influence of external factors, and he follows KLEBS in holding that the ordinary

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[2] GOEBEL, K., Einleitung in die experimentelle Morphologie der Pflanzen. 8vo. pp. vi + 260. *figs. 135*. Leipzig and Berlin: B. G. Teubner. 1908. *M* 8.

succession of events in the life-history of a plant is no more normal than any other succession of events that may be produced by altering the external conditions. Like KLEBS, therefore, he is obliged to discard the word normal or place it in quotation marks. To the reviewer such a viewpoint seems fundamentally sound, and it has the added attraction of throwing open to experimentation all the phenomena of development, including those that have been referred to heredity or mysterious internal causes. In harmony with this fundamental principle, illustrations are given of the omission of individual stages from a developmental series, the transposition of two such stages, and the retention of a given stage, if the conditions favorable to another are not forthcoming; even the juvenile stages may be retained through life in many instances, if the external conditions are favorable thereto. On the other hand, there are cases where juvenile forms bear flowers, the intervening stages being skipped.

The body of the book consists of four chapters, dealing respectively with the influence of external and internal conditions on leaf form, the conditions determining variations in the development of main and lateral axes, regeneration, and polarity. In all of these chapters there is a wealth of detail of the utmost value to students in experimental morphology and to those desiring a bibliography of these subjects. Not only is the literature summarized, but there are many new and suggestive experiments mentioned here for the first time. In the chapter on leaf form, heterophylly in xerophytic species of Veronica and in such amphibious plants as Limnophila plays an important part. Mutation in some ferns is definitely referred to the operation of external factors. Plants with orthotropous and plagiotropous shoots are found to belong to two categories. For example, lateral branches of the spruce (*Picea excelsa*) become orthotropous if the terminal shoot is removed, while such a reaction does not occur in Araucaria; the former illustrates lability, the latter stability. The same chapter contains some very interesting data concerning variation in flowers and inflorescences. Of especial significance is the transformation of Selaginella "flowers" into vegetative shoots by introducing the plants into moist air. Variations in flower color and form, and the relations of cleistogamous and chasmogamous flowers are here considered, much credit being properly given to KLEBS for his path-breaking work. The chapters on regeneration and polarity are most useful in gathering together the scattered literature, as well as in adding new facts.

Although the volume is denominated an introduction and not a handbook, it is indispensable not only to experimental morphologists, bnt to all morphologists, physiologists, and ecologists, who are at all interested in the origin and significance of plant structures. To the reviewer the book is a bit disappointing, because it seems not to get down to fundamental principles. Throughout the the work nutrition is regarded as a dominant external factor. While it is true that it is as yet impossible to resolve nutrition into its components, it is at least better to attempt to do so than to assume that nutrition is really anything definite, or even ultimate. To many of us the work will be more useful as a summary of facts than as an explanation of the conditions underlying them.—HENRY C. COWLES.

The vegetation of Switzerland

CHRIST'S *Das Pflanzenleben der Schweiz* was published in 1879. A French translation of this work entitled *La flore de la Suisse et ses origines* appeared in 1907.[3] An apology is due the author and publishers for the tardiness of this review. The translator is E. TIÈCHE, and the author has added a supplement in which he summarizes the geobotanical work that has been done since the publication of the first edition. As the original work has never been reviewed in the GAZETTE, a summary of its contents may be acceptable.

After a brief discussion of the fundamental principles of plant distribution, especially the influence of climate, and migrations, the vegetational regions of Switzerland are described in great detail. The primary divisions are naturally zonal in form, since the country is so largely mountainous. The author enumerrates four zones: (1) the basal zone, having considerable likeness in its flora to the Mediterranean region, and largely under cultivation, with the grape as the characteristic culture plant; (2) the zone of deciduous forest, dominated in the south by the chestnut, in the north by the beech; (3) the zone of coniferous forest, composed of spruce, fir, larch, and Cembran pine; (4) the alpine region, to which a third of the discussion is devoted. Here particularly the author considers the problems of migration, tracing an important element among the alpine plants to the mountains of north-central Asia as their point of origin. The large endemic element of the alpine flora is also discussed at some length.

The work is characterized by minuteness of detail, possible because of the thoroughness with which the Alps have been explored and studied by botanists. Such a work is hardly yet possible in any portion of our country.

The supplement, which is a résumé of the work of recent students, adds nothing of general importance, but merely fills out details here and there. It would seem that in view of the great advance that has been made in the study of plant geography during the thirty years since the appearance of the first edition, a better plan would have been to rewrite the whole.—WILLIAM S. COOPER.

MINOR NOTICES

Local tree floras.—RAMALEY has published a thin volume[4] whose really useful part consists of descriptions of the trees of Colorado, with analytic keys and a considerable number of illustrations. These will be helpful to those who wish to study the woody plants. This part is prefaced by an essay of some 30 pages on the "wild flowers" of the state, with many halftones of vegetation and scenery, and a number of good outline drawings. The title, *Wild flowers and trees of Colorado*, is thus literally justified, but practically it is misleading. We hoped from the announcement to see a popular flora embracing the commoner

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[3] CHRIST, H., La flore de la Suisse et ses origines. pp. xiv+571+119. *pls. 4. maps 5.* Bâle-Geneva-Lyons: Georg & Cie. 1907.

[4] RAMALEY, F., Wild flowers and trees of Colorado. 8vo. pp. vi+78. *figs. 70.* Boulder, Colo.: A. A. Greenman. 1909.

plants of the state, after the fashion of some of the excellent books on the eastern flora. The demand for this sort of a book, we hope, will induce Dr. RAMALEY to prepare such a volume. The tourist travel alone would doubtless quickly absorb a reasonable edition.

MACKENSEN, a teacher in the San Antonio (Texas) High School, has published, as well as written, a handy a little pamphlet[5] on "all the woody plants growing naturally within five or six miles of the center of the city of San Antonio." According to the author's investigations there are just one hundred such species, of which at least ten are introduced. This number of species is surprisingly large, but is due to the author's lax conception of woody plants, for we find included various vines, agave, and the cactuses, not to mention a herbaceous mallow described as "woody below." The descriptions are untechnical, and there are some interesting observations on certain species. The lack of a key will limit the usefulness of the pamphlet.—C. R. B.

Asiatic palms.—The present volume, a monographic presentation of the species of Calamus,[6] records the results obtained by a careful specialist after years of study of living plants in their native habitat and the critical examination and comparison of dried specimens from the larger herbaria of the world. The subject-matter is arranged under ten headings, the first being an introductory essay of forty-five pages giving a detailed discussion of all parts of the plants entering in any way into their classification, the treatment of the genus by former authors, present limitations, especially with reference to Daemonorops and Palmijuncus, and finally the geographical distribution of species.

The author then introduces the taxonomy by giving a systematic conspectus of the species, arranged in sixteen groups, based primarily on the presence or absence of leaf-cirri, and further on the characters of the leaf sheaths and inflorescence; the conspectus is followed by a synopsis of the species (brief characterizations of the 201 recognized species); and lastly their enumeration with bibliography, detailed descriptions, and copious notes. With the exception of five species, known only from RUMPH'S descriptions and figures, all of those treated in the text have been beautifully illustrated by natural-size phototype reproductions from the author's own negatives. A complete index to species and plates concludes the volume. The work is an important contribution to taxonomic literature.—J. M. GREENMAN.

A forcing process.—JOHANNSEN'S process of forcing plants by treatment with ether vapor has been of considerable service in the commercial production of unseasonable flowers. A much simpler and in every way more practicable

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[5] MACKENSEN, B., The trees and shrubs of San Antonio and vicinity: a handbook of the woody plants growing naturally in and about San Antonio, Texas. 12mo. paper. pp. 51. *pls. 12.* San Antonio: The author. 1909.

[6] BECCARI, ODOARDO, Asiatic palms—Lepidocaryeae. Part I. The species of Calamus. Ann. Roy. Bot. Gard. Calcutta 11:1–518. *pls. 238.* 1908.

process is described by MOLISCH in a little pamphlet, entitled *Das Warmbad.*[7] The treatment consists merely in immersing the shoots of potted plants (by inversion) in water of 30–35° C. for 9–12 hours and then keeping them in a warm (25°), moist, dark chamber until the leaves begin to appear, when further development proceeds under ordinary greenhouse conditions. Lilacs and spiraeas treated to the warm bath about mid-November flowered before Christmas, and azaleas early in January; while untreated plants, under the same conditions, had at this time hardly started their buds. The exact duration and temperature of the bath for securing the best results vary with different species and races. The process is already in use and is likely to be extensively practiced.—C. R. B.

The flora of Korea.—The first part of this work[8] includes the families Ranunculaceae to Dipsaceae, and their sequence is essentially that of BENTHAM and HOOKER in the *Genera plantarum*. Concise dichotomous keys to the genera introduce each family containing more than one genus, and similar keys at the beginning of each genus precede the enumeration of species. Under the species a very full bibliography is given, as well as the citation of exsiccatae and the general distribution. The Japanese name is also given in many cases. Several new species and varieties are published, and the text is augmented by fifteen full-page clean-cut illustrations. An index to genera mentioned in the flora concludes the part.—J. M. GREENMAN.

North American Flora.—Volume XVII, Part I, of this work[9] contains the following groups: Typhaceae by P. WILSON; Sparganiaceae, Elodeaceae, and Hydrocharitaceae by P. A. RYDBERG; Zannichelliaceae, Zosteraceae, Cymodoraceae, Naiadaceae, and Lilaeaceae by N. TAYLOR; Scheuchzeriaceae by N. L. BRITTON; Alismaceae by J. K. SMALL; Butomaceae, Poaceae (pars) by G. V. NASH. New species are described in the following genera: Sparganium (2), Echinodorus (1), and Trachypogon (2). One new genus (*Machaerocarpus*) of the Alismaceae is proposed, being based on *Damasonium californicum* Torr.—J. M. GREENMAN.

Diseases of trees.—A bulletin, embodying the results of a number of years' investigation of some of the more important diseases of deciduous trees by VON SCHRENK and SPAULDING, has been issued by the national Bureau of Plant Industry.[10] The bulletin contains a large amount of information (there is unfortunately little available) on diseases due to environment, to wound fungi (far

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[7] MOLISCH, H., Das Warmbad als Mittel zum Treiben der Pflanzen. 8vo. pp. 38. *figs. 12.* Jena: Gustav Fischer. 1909. *M* 1.20.

[8] NAKAI, T., Flora Koreana. Pars Prima. Jour. Sci. Coll. **26**: 1–304. *pls. 1–15.* 1909.

[9] North American Flora, Vol. XVII, Part I, pp. 1–98. New York Botanical Garden, 1909.

[10] SCHRENK, H. VON, AND SPAULDING, P., Diseases of deciduous forest trees. Bur. Pl. Ind. U. S. Dept. Agric. Bull. 149. pp. 85. *pls. 10. figs. 11.* 1909.

the largest category), and to miscellaneous parasites and saprophytes, with some discussion of the decay of structural timbers. The Polyporaceae are the greatest devastators of our forests. A useful bibliography is appended.—C. R. B.

Vegetationsbilder.—The third part of the seventh series of KARSTEN and SCHENCK'S well-known work[11] presents six plates of the vegetation of the moors, *Bockser* (high plains with dry grasses and sedges), and forest of the northern *Schwarzwald*, with text, by OTTO FEUCHT; the fourth illustrates the sea strand, littoral, sublittoral, and submontane formations on the Dalmatian coast, with text by L. ADAMOVIC; while the fifth pictures the various curious plants characteristic of the Abyssinian highlands.—C. R. B.

NOTES FOR STUDENTS

Taxonomic notes.—O. BECCARI (Phil. Jour. Sci. **3**:339–342. 1908) has described 3 new species and 2 new varieties of ferns from the Philippine Islands.—R. C. BENEDICT (Bull. Torr. Bot. Club **36**:41–49. 1909) records 4 new hybrids in the genus Dryopteris from eastern America.—C. CHRISTENSEN (Rep. Nov. Sp. **6**:380, 381. 1909) records a new species of Dryopteris from Brazil.—A. COGNIAUX (*ibid.* 304–307) publishes 5 new species of orchids from Jamaica.—F. S. COLLINS (Rhodora **11**:17–20. *pl. 78.* 1909) has published 4 new species of the genus Cladophora, and (*ibid.* 23–26) under "Notes on Monostroma" records a new form of *Monostroma orbiculatum* from Massachusetts.—E. B. COPELAND (Phil. Jour. Sci. **3**:343–357. *pls. 1–8.* 1908), under the titles "New genera and species of Bornean ferns," and "New species of Cyathea," has published 20 new species and 2 new genera (*Macroglossum* and *Phanerosorus*).—L. A. DODE (Bull. Soc. Bot. Fr. IV. **8**:648–656. 1908) describes 12 new species and 3 new hybrids of trees and shrubs; these include a Robinia from Colorado and a Salix from New Jersey.—F. EICHLAM (Monats. Kakteenk. **19**:1–5. 1909) characterizes a new species and 4 varieties of Mamillaria from Guatemala, and (*ibid.* 22–25) describes a new species of Pereskiopsis from the same general region.—A. D. E. ELMER (Leafl. Phil. Bot. **2**:445–594. 1908–1909) has described 101 new species and 3 varieties of Philippine plants. A new genus (*Elmeria* Ridl.) of the Zingiberaceae is proposed, and a synopsis of the genus Rubus is given, in which the author recognizes 17 species for the Philippine Islands, 3 being new to science.—A. ENGLER (Bot. Jahrb. **43**:161–198. 1909) has published new species of African plants as follows: 10 in the Olacaceae, 11 in the Opiliaceae, 2 in the Octoknemataceae, 11 in the Icacinaceae, and 13 in the Aizoaceae.—E. GILG (*ibid.* 97–128), in an article entitled "Balsaminaceae africanae," recognizes 85 species of Impatiens from Africa, and of these 26 are new to science.—M. GÜRKE (*ibid.* 199, 200) records 3 new species of Ebenaceae from Africa, and (Monats. Kakteenk. **19**: 12–14. 1909) describes *Rhipsalis Novaesii* and accompanies the description by illustrations of the flower; the plant is a native of Brazil.—W. HERTER (Bot.

[11] KARSTEN, G., AND SCHENCK, H., Vegetationsbilder. Series vii, parts 3–5. text and *pls. 13–30.* 4to. Jena: Gustav Fischer. 1909. *M* 4 per part.

Jahrb. **43**: Beibl. No. 98, pp. 1–56), under the title "Beiträge zur Kenntniss der Gattung Lycopodium," has published 48 new species, of which 30 are from the West Indies, Mexico, Central and South America.—J. D. HOOKER (Kew Bull. 1–12. 1909) presents the results of recent studies in the genus Impatiens from Indo-China and the Malayan Peninsula, recognizing for this region 27 species, of which 5 are new to science; a key precedes the enumeration of species.—M. A. HOWE (Bull. Torr. Bot. Club **36**:75–104. 1909) has published 7 new species of the Siphonales, and presents also a synoptical key to the living species of Neomeris.—F. KRÄNZLEIN (Rep. Nov. Sp. **6**:317. 1909) records a new species of Dendrobium from the Philippine Islands.—K. KRAUSE (Bot. Jahrb. **43**:129–160. 1909) has published 40 new species of Rubiaceae from Africa.—E. D. MERRILL (Phil. Journ. Sci. **3**:359–358. 1908) gives a synopsis of the Philippine species of Garcinia, recognizing 17 species, of which 5 are new; the same author (*ibid.* 369–382), under the title "Philippine Ericaceae," presents a consideration of the four genera representing this family in the Philippines, and accompanies Vaccinium (19 species) and Rhododendron (16 species) with determinative keys, characterizing 3 species and one variety as new; further, he gives (*ibid.* 385–442) a list of plants from the Batanes and Babuyanes Islands, describing 16 new species.—E. PAX (Bot. Jahrb. **43**:218–224. 1909), under the title "Euphorbiaceae africanae X," has published 15 species new to science, and proposes 2 new genera (*Holstia* and *Neopycnocoma*); the same author (*ibid.* 75–90) describes 43 species and 4 varieties of Euphorbiaceae as new to science.—J. PERKINS (*ibid.* 214–217) publishes jointly with E. GILG a new genus (*Afrostyrax*) of the Styracaceae; the genus is represented by one species, *A. kamerunensis*, from Kamerun.—R. PILGER (*ibid.* 91–96), under the heading "Gramineae africanae VIII," describes 8 new species of grasses.—J. A. PURPUS (Monats. Kakteenk. **19**:38–41. 1909) describes and illustrates a new species of Cereus from Mexico.—B. L. ROBINSON and M. L. FERNALD (Rhodora **11**:33–61. 1909), under the title "Emendations of the seventh edition of Gray's Manual I," has given an annotated list of corrections, additions, extension of ranges, etc., and includes a new species of Callirhoë from Missouri.—R. A. ROLFE (Kew Bull. 61–66. 1909), in an article entitled "New Orchids: Decade 33," publishes 10 new species, of which 4 are American.—E. ROSENSTOCK (Rep. Nov. Sp. **6**:308–316. 1909) has published 9 species and 4 varieties of ferns as new to science; these are based on collections made by Dr. O. BUCHTIEN in Bolivia.—Different authors (*ibid.* 341–352), under the title "Ex herbario Hassleriano: Novitates paraguarienses I," have published 12 species and 16 varieties of flowering plants as new to science.—F. J. SEAVER (Mycologia **1**:41–76. *pls. 4, 5.* 1909) gives a synopsis of the Nectrieae of North America, recognizing 11 genera and about 40 species, 2 of which are here described for the first time; to the genus Nectria 20 species are referred, and a determinative key precedes their enumeration.—P. C. STANDLEY (Muhlenbergia **5**:46, 47. 1909) proposes a new species of Castilleja from Arizona.—W. WEINGART (Monats. Kakteenk. **19**:17–22. 1909) describes and illustrates a new species of Cereus from Paraguay.—E. O. WOOTON

and P. C. STANDLEY (Bull. Torr. Bot. Club **36**: 105–112. 1909) have described 11 species and one variety of flowering plants as new to science; these are based chiefly on the collections made in Mexico by O. B. METCALF.—J. M. GREENMAN.

Morphology of Monascus.—BARKER[12] and OLIVE[13] in their investigations of Monascus describe the ascogenous hyphae as arising from an oogonium, which has been fertilized by an antheridium, thus establishing a true sexual process for this form, while KUYPER[14] denies any fusion of sexual cells. IKENO[15] asserts that there are no ascogenous hyphae in Monascus, but that uninucleate cytoplasmic masses arise by free cell formation from the ascogonium, which in turn divide also by free cell formation to form the spores. KUYPER in the main agrees with IKENO as to the absence of sexual cells at the origin of the perithecium, while BARKER and OLIVE hold that the asci arise from the cells of the ascogenous hyphae, which arise from an ascogonium. DANGEARD,[16] however, regards BARKER'S antheridium as a nourishing cell for the ascogonium. According to DANGEARD the ascogonium divides into several cells, each of which gives rise to binucleate cells, which form the asci and whose nuclei fuse.

SCHIKORRA[17] has recently investigated pure cultures of two species of Monascus, *M. purpureus* Went. and an undetermined species, and believes that he has explained the inconsistencies of the above-mentioned authors. The life-history of both these species is similar. The female organ arises as a multinucleate subterminal cell of a hypha. This cell later divides into a trichogyne and oogonium. The apical cell is a multinucleate antheridium and fuses with the trichogyne, into which the male nuclei pass by means of a pore. There is an evident gap in observations at this point and the author next describes the male and female nuclei as arranged in pairs in the ascogonium. From the hypha below the sexual organs investing hyphae arise, which form a two-layered perithecial wall. Ascogenous hyphae originate from the fertilized oogonium. These hyphae contain nuclei in pairs, which multiply by conjugate divisions, although no such divisions are figured. The asci are formed from the subterminal cells

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[12] BARKER, P. T. B., The morphology and development of the ascocarp of Monascus. Annals of Botany **17**: 167–236. 1903.

[13] OLIVE, E. W., The morphology of *Monascus purpureus*. BOT. GAZETTE **39**: 50–60. 1905.

[14] KUYPER, H. P., Die Peritheciumentwicklung von *Monascus purpureus* Went. und *Monascus Barkeri* Dang., so wie die systematische Stellung dieser Pilze. Ann. Mycol. **3**: 32–81. 1905.

[15] IKENO, S., Ueber die Sporenbildung und systematische Stellung von *Monascus purpureus* Went. Ber. Deutsch. Bot. Gesells. **21**: 259–269. 1903.

[16] DANGEARD, P. A., Recherches sur développement du périthèce chez les Ascomycètes. Le Botaniste **10**: 177, etc. 1907.

[17] SCHIKORRA, W., Ueber die Entwicklungsgeschichte von Monascus. Zeits. Bot. **1**: 379–410. 1909.

of the recurved tips of these ascogenous hyphae. The ascus contains two nuclei, which fuse, and this fusion nucleus divides by a triple division to form the eight nuclei of the ascospores. The two ascus nuclei are looked upon as male and female, and their fusion completes the union of the gametes which initiated the development of the perithecium. The method of spore formation was not made out. The ascus walls finally degenerate, leaving the spores free in the perithecium.

From his studies SCHIKORRA concludes that Monascus is a true ascomycete, belonging to the family Aspergillaceae, in the order Plectascineae. It is placed here on account of the similarity of its perithecium to that of Penicillium and Aspergillus.

The presence of a true sexual process in the Ascomycetes has been established beyond a doubt in a considerable number of forms. The investigations of several species have shown, however, that a normal sexual process is not to be expected in all. The apparent absence of any nuclear fusion in the fertilized oogonium, although the sexual nuclei become arranged in pairs, brings Monascus into harmony with CLAUSSEN'S results on Pyronema, in which nuclear fusion is claimed to occur only in the ascus, a sort of modified sexual process, therefore, being present. Realizing how difficult it is observe nuclear fusion in the oogonium, on account of the small size of the nuclei and the apparent rapidity of the process, one cannot but feel that the actual fusion in the ascogonium may have been overlooked. CLAUSSEN'S and SCHIKORRA'S results support somewhat MAIRE'S contention that there is a tendency in the Ascomycetes to form a synkaryophyte analogous to that of the Basidiomycetes.—J. B. OVERTON.

Electricity and photosynthesis.—KOLTONSKI publishes an elaborate report[18] of experiments on the effects of electric currents of varying strength, density, and direction upon the number of gas bubbles given off by Elodea and Ceratophyllum in unit time. The work is an extension of that of THOUVENIN,[19] and proceeds, like his, upon the assumption (which may usually be valid) that under normal conditions the number of these bubbles constitutes a fair measure of the rate of photosynthesis; but it is hardly necessary to say that the action of an electric current may create conditions which render this assumption invalid. It is interesting to know that electric currents produce an effect upon the evolution of gases in these plants; but it is impossible to interpret KOLTONSKI'S results. He presents them, indeed, as only a starting-point for further investigations.—C. R. B.

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[8] KOLTONSKI, A., Ueber den Einfluss der elektrischen Ströme auf die Kohlensäureassimilation der Wasserpflanzen. Beih. Bot. Centralbl. **23**: 204–271. *figs.* 4. 1909.

[9] THOUVENIN, M., De l'influence des courants électriques continus sur la décomposition de l'acide carbonique chez les végétaux aquatiques. Rev. Gén. Bot. **8**: 433–450. 1896.

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THE BOTANICAL GAZETTE

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BOTANICAL GAZETTE

SEPTEMBER 1909

PRELIMINARY ACCOUNT OF THE OVULE, GAMETOPHYTES, AND EMBRYO OF *WIDDRINGTONIA CUPRESSOIDES*

W. T. SAXTON

(WITH PLATE XI AND THREE FIGURES)

INTRODUCTION

The genus Widdringtonia contains about six species of trees in equatorial and south Africa and Madagascar. A plant from tropical Africa figured by GOEBEL (**16**) and by EICHLER in ENGLER and PRANTL (**12**) as *Callitris quadrivalvis* is probably also very closely related to Widdringtonia, but differs in the much smaller number of ovules and also to some extent in the foliage, and was separated from that genus by MASTERS (**26**) as *Tetraclinis articulata*. I have not had an opportunity to examine this plant.

The genus Callitris has unfortunately been repeatedly confused with Widdringtonia, and the species of the latter are included under the former genus by BENTHAM and HOOKER (**2**), EICHLER (**12**), JACKSON (**17**), BOLUS and WOLLEY-DOD (**3**), and MARLOTH (**25**). Widdringtonia was clearly distinguished as a genus, however, by ENDLICHER (**13**), and is recognized by such authorities as MASTERS (**26, 27**) and RENDLE (**30**), as well as by SIM (**34**) and MAIDEN (**24**), the chief authorities on the forest floras of South Africa and Australia respectively. The two genera differ widely in cones and foliage, and while Widdringtonia (excluding fossil species) is restricted to south and central Africa and Madagascar, Callitris is as rigidly restricted to the Australian region. I have been fortunate in having a good opportunity to study several species of both genera growing in the Government Plantations at Tokai, the majority of the trees

coning freely, and am confident that no one who took advantage of a similar opportunity would still wish to unite the two genera.

Only two species of conifers occur native in the Cape Peninsula, and both are confined to the tops and upper slopes of the mountains; these are *Podocarpus Thunbergii* Hook. and *Widdringtonia cupressoides* Endl. (**3**). The development of Podocarpus is fairly well known, having been studied in other species (COKER **6**, BURLINGAME **4**), but that of Widdringtonia (excluding Tetraclinis) is entirely unknown so far as I am aware.

In attempting to work out the life-history the chief difficulty has been the collection of the material. The plant occurs only at an altitude of about 2000 feet and upward, and it has always required at least four or five hours to obtain a single collection. Furthermore, a considerable portion of the cones contain only abortive ovules, especially in certain localities, and this has often made the collection of even a small number of ovules very tedious. As a consequence it has only been possible to make collections at rather long intervals, and these have each only included a small number of ovules, particularly in the later stages. As these difficulties will again be encountered in trying to fill in the gaps in the present account, it is thought best to publish the results now presented. Here and there comparisons are made with Callitris, the development of which is very similar. I hope later to be able to study this genus more in detail.

I am glad to take this opportunity of thanking the authorities at Tokai for permission to collect cones of all species of Widdringtonia and Callitris grown in the Government Plantations, and also my friend Mr. E. P. PHILLIPS, for kindly collecting and fixing material on three occasions.

METHODS

The material has in almost all cases been fixed in the field, and all figures are drawn from such material. Various fixing agents have been tried, including different strengths of chromacetic acid with and without osmic acid, but the following has been found the most generally useful (CHAMBERLAIN **5**, p. 20): picric acid (saturated solution in 50 per cent. alcohol), 100^{cc}; glacial acetic acid, 5^{cc};

corrosive sublimate, 5^{gm}.[1] Material was fixed for 24 hours and then washed in 50 per cent. alcohol until the alcohol was no longer colored yellow (a week or more as a rule), then 12–24 hours in 75 per cent., 85 per cent., and 94 per cent. alcohol, and at least 60 hours in absolute alcohol changed three or more times. Both xylol and cedar oil (in 25 per cent. grades with alcohol) were tried to precede infiltration with paraffin, but the former was entirely discarded after a few trials. Material was taken from cedar oil through 25 per cent., 50 per cent., and 75 per cent. solutions of soft paraffin in cedar oil, to pure paraffin, melting point 48° C., using tiny wire-gauze baskets, for the purpose, as described by FERGUSON (**14**), and allowing 48 hours in each solution. At least 48 hours longer was allowed in two fresh lots of soft paraffin (an indication of the time required here is given by the time taken to wash out the fixing agent), 12–24 hours in a mixture of soft and hard paraffin, and finally 24 hours in hard paraffin, melting point 55° C., in which it was imbedded. These very long periods in the oven were found to be absolutely necessary to insure proper infiltration of the paraffin.

In young stages the ovules were fixed whole, or when very young a small part of the tissue of the cone was cut off bearing the ovules. Later the integument becomes too hard to cut, and it was necessary to dissect out the nucellus, a process which can be carried out fairly easily in practice. In some stages after fertilization the prothallus was dissected out, and where nearly mature embryos were present these were fixed alone. The staminate cones were fixed whole, being very small, but required even longer periods in the oven than those mentioned above.

The stains were (1) Delafield's hematoxylin diluted 8–10 times, allowed to stain 4–12 hours, extracted with very dilute acid (aqueous), and followed by a thorough washing in water; and (2) Flemming's triple stain. The best results were obtained with the former, especially after the fixing agent mentioned above. It appears to be always the case that the very best staining effects of Delafield's hematoxylin are obtained after a fixing agent containing corrosive sublimate. The value of this hematoxylin as a nuclear stain has not at present been realized.

[1] I am indebted to Mr. A. J. BALLANTINE for suggesting the use of this reagent.

DESCRIPTION

The youngest ovulate cones seen were about 3 or 4^{mm} across, when the two decussate pairs of scales were widely spreading. The ovules are generally 20–30 in number and appear to be evenly distributed over the broadened end of the axis. The subsequent development proves this position to be only apparent, and that they are actually situated on the fused bases of the scales. By considerable growth of the basal part of the scales the ovules are carried farther apart, and after the former have grown together the latter are found on the sides of the ridges where the lower and upper scales meet.

The youngest ovule found is shown in *fig. 1*. The comparatively long tubular micropyle is very noticeable even at this early stage; the upper cells are already dead and empty and growth of the integument proceeds only at its base. The layer of small cells with dense contents lining the basal part of the micropyle grow actively some time after pollination and narrow considerably the micropylar opening at this part. Apparently, however, the micropyle is never completely closed by this means, but only by the accumulation of dust, etc., at its apex. The integument so soon becomes too hard to section satisfactorily that it is impossible to speak with confidence on this point.

The staminate cones are mature at about the same time that the ovules are found in this condition. They are very small and so inconspicuous until they change color at or near maturity that I have never succeeded in collecting immature cones with a view to following the development of the microspores. The sporophylls are peltate and somewhat pointed toward the apex of the cone and bear five pollen sacs abaxially placed on the stalk, the wall of the microsporangia being only one cell thick (*fig. 2*). The pollen grains have an exceptionally thick cell wall, and only a single nucleus can be distinguished in the mature grain, and in early stages of germination (*figs. 3, 4*). It seems usual in the Cupressineae for no prothallial cell to be formed in the male gametophyte, COKER (8) having noted their absence in eight or nine genera, including Callitris. It is of course impossible to assert their absence in Widdringtonia without obtaining preparations of microspores of various ages, but it may be assumed that none is present. As a large number of pollen grains have been

examined in the stages here figured, it seems scarcely possible that a second nucleus can be present, but it is unusual for the division giving rise to the generative and tube nuclei to be delayed so long. COKER (**8**) reports that this division occurs after shedding in certain cases, but says that it occurs before shedding in *Callitris* sp. As will be seen, however, the whole history of the male gametophyte shows a minimum of nuclear activity.

The pollen grain has a fairly thin cellulose endospore and a very thick and rather hard exospore (*fig. 4*), and on germination (as shown in the figure) the exospore alone begins to grow, without any immediate growth of the endospore or any trace of tube formation. The number of pollen grains which begin to germinate in each ovule varies from one to four, three being the most usual number (*fig. 3*). The ovule here figured closely resembles that of *fig. 1*, and therefore only a few of the cells have been drawn.

No trace of a megaspore mother cell or cells can be seen in ovules of this age, and stages to illustrate megasporogenesis are unfortunately wanting at present. GOEBEL (**16**) figures an ovule of Tetraclinis (*Callitris quadrivalvis*) in which a considerable number of what are probably megaspore mother cells are figured. Several ovules considerably older than that just mentioned show the nucellus clearly differentiated into peripheral and central regions. This central region and the innermost cells of the peripheral region are represented in *fig. 5*. The cells of the central part are somewhat larger and are characterized by having only a very scanty supply of cytoplasm. Probably these cells are a large group of megaspores, one of which later grows to form the prothallus. In this case GOEBEL'S figure of Tetraclinis would doubtless also serve to illustrate the corresponding structure in Widdringtonia.

It is certain in any case that normally only one megaspore develops, since no "secondary prothalli" have been found, as described by LAWSON (**20**) in *Sequoia sempervirens*. An ovule of *Callitris verrucosa*, however, has been sectioned, which contained two secondary prothalli (*text fig. 1*). In the next stage figured the very large prothallus is already formed. The embryo sac is lined with cytoplasm containing a single layer of free nuclei and bounding one large central vacuole. *Fig. 6* is a sketch of a whole ovule in

optical section at this stage, showing the winged integument (the wings are only one cell thick), nucellus, and prothallus. The long tubular micropyle is now very noticeable. The ovules are nearly all curved like this one, so that there is only one plane of symmetry in the ovule. Occasionally a second curvature is found at right angles to this one, in which case longitudinal sections are apt to be puzzling unless a whole series is carefully studied. Cones containing ovules of about the age shown in *fig. 6* are growing on the middle branch in the accompanying photograph (*text fig. 2*). Those on the right have just been pollinated and those on the left have young embryos, all being gathered February 28, 1909.

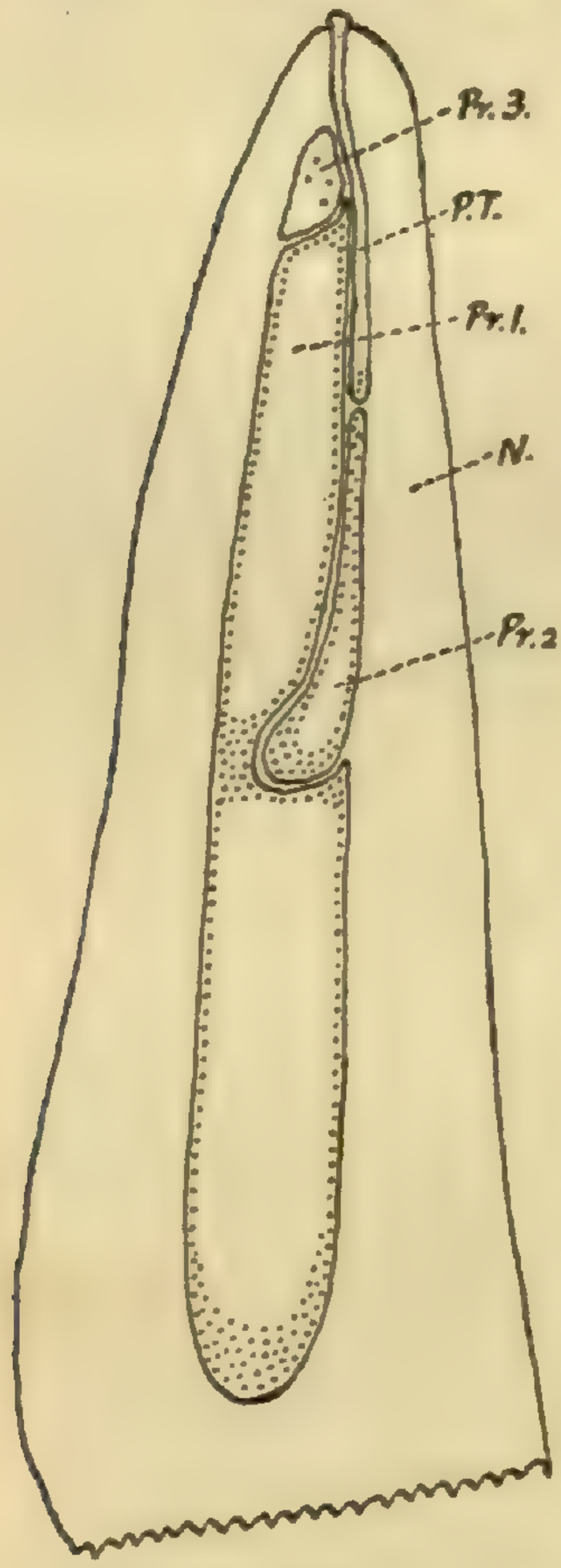

FIG. 1.—Median longitudinal section of nucellus, *n*, of *Callitris verrucosa: pt*, pollen tube; *pr*¹, primary prothallus; *pr*², *pr*³, secondary prothalli. ×40.

Fig. 7 shows the apex of a nucellus about the same age as that in *fig. 6*. Both preparations indicate that only a single pollen tube is present and a case is only rarely met with in which more than one pollen tube develops; though, as seen above, three usually begin to germinate. Only two very small nuclei are found in the pollen tube of this age, no doubt the tube and generative nuclei.

Fig. 8 represents a very small part of the prothallus with its lining layer of cytoplasm and two of the free nuclei. In some preparations, such as the one figured, the nuclei can be seen to be paired, probably indicating that a simultaneous division has recently taken place. None of these divisions have been seen, but if, as is probable, they occur always very rapidly and simultaneously there might be only about ten separate periods of an hour or two each, extending over three months or more, during which the requisite stages could be obtained. It may also be that the time of day or night at which these divisions occur is always about the same, though there is no evidence that this is so with other divisions; at any rate

in the sporophyte, collections at 9 A. M., 12 noon, and 6 or 7 P. M. show an approximately similar number of nuclear figures.

It is noticeable in *fig. 8* that the nuclei project slightly into the vacuole. Later on the layer of cytoplasm thickens considerably and the nuclei are then completely sunk in it. *Figs. 9* and *10* indicate the principal changes met with after the last stage figured. *Fig. 9* shows clearly the process of wall formation in the prothallus,

FIG. 2.—*Widdringtonia cupressoides* (see text). ×$\frac{1}{6}$.

alveoli being organized as described by SOKOLOWA (**35**) and other writers for various gymnosperms. The cells at first formed are invariably uninucleate, and the original cell walls persist. The binucleate and multinucleate condition met with later (see below) does not arise, therefore, in the same way as the binucleate prothallus cells of Cryptomeria described by LAWSON (**21**). The pollen tube has meanwhile reached the tip of the prothallus, and even before wall formation begins, it penetrates the megaspore membrane and grows down just inside of it, on or near the surface of the prothallus, to about one-third or one-half way down.

Fig. 10 is drawn from the same series of sections as *fig. 9* and represents the tip of the pollen tube which has in this way pene-

trated nearly half-way down the prothallus. Three nuclei are now met with in the pollen tube, all imbedded in a rather dense mass of cytoplasm. One nucleus is considerably larger than the other and is clearly differentiated from the cytoplasm. The two smaller (stalk and the tube nuclei), on the other hand, are only with difficulty distinguished either in structure or staining capacity from the surrounding cytoplasm. The larger nucleus with its cytoplasm is evidently the body cell, and its structure is shown in detail in *fig.* 11.

Fig. 12 is a sketch of a longitudinal section of the upper half of a nucellus at a slightly later stage, when cell division in the prothallus is just completed. The tube with its conspicuous body cell can be seen in the position described above. The end of this pollen tube is shown in detail in *fig.* 13; and no nucleus except that of the body cell can now be found. A careful and repeated search in this and adjoining sections of the series (which is quite complete) failed to reveal any trace of the tube and stalk nuclei, even with an oil-immersion objective. Unfortunately the tube figured was the only one showing this stage (all others were before cell division in the prothallus was complete), and the figure will probably therefore be regarded as abnormal; but the whole structure of the ovule and pollen tube seemed perfectly normal in other respects, the fixation was entirely satisfactory, as was the staining, and there was not the least indication that any part of the tube or its contents had washed off the slide during staining, etc. The staining reactions of the tube and stalk nuclei of *fig.* 10, together with their absence in *fig.* 13, indicate that they break down completely and that their substance becomes blended with the cytoplasm.

The cytological characters of the body cell nucleus are indicated in *fig.* 14, and a comparison with the nucleus in *fig.* 11 suggests that division will shortly take place, giving rise to two sperm nuclei. Up to this point no trace of archegonia or archegonium initials can be seen.

Fig. 15 represents the upper half of the prothallus (and part of the nucellus) at a considerably later stage, after the archegonia are mature and fertilization has been effected.[2] The distribution of the archegonia is very remarkable and recalls that described for *Sequoia*

[2] I was unfortunately unable to make any collections for a number of weeks at this time.

sempervirens by ARNOLDI (**1**) and LAWSON (**20**). Their distribution is evidently determined largely by the position of the pollen tube. Thirty-eight archegonia are shown in this figure, but only those are indicated parts of which at least could be seen in a single section; the total number is slightly over fifty. Of several prothalli collected on the same date, one other showed an almost identical structure, but the rest were somewhat older and the archegonia were disorganized. There was an indication, however, that the number of archegonia in these other prothalli was considerably less, though their distribution must have been essentially similar.

The lowest group of archegonia from *fig. 15* has been drawn on a larger scale in *fig. 16*. Of these archegonia the lowest but one and the lowest but three have evidently been fertilized, as each contains a proembryo. This fact, together with the presence of a single pollen tube, indicates that, as is usual in Cupressineae (LAWSON **23**), two sperm cells are organized and both are functional. However, only indirect evidence is available in the present case. Unfortunately the unfertilized archegonia of the group are not in very good condition, having doubtless been organized some time previously and being about to disintegrate; hence it is quite possible that the details exhibited are to some extent different from those of a recently formed archegonium.

Only the lowest archegonium of the group has any trace of what might be interpreted as neck cells, and even this cell (shaded in the figure) is probably only a prothallus cell which happens to lie immediately over the archegonium. It is quite clear that the archegonia arise from cells deep in the prothallus, and possibly this may be the reason why no neck cells are formed, though LAWSON (**20**) records them in Sequoia; but in that genus the archegonia grow in such a way as to push their necks to the surface, whereas in Widdringtonia they remain deep-seated in the prothallus. It may be that the neck cells disintegrate entirely and leave no trace, and this would be the natural explanation in the case of fertilized archegonia, as noted by KILDAHL (**18**) in connection with a similar absence of neck cells in *Phyllocladus alpinus;* there is no apparent reason, however, to suppose that the neck cells have completely disappeared in all the unfertilized archegonia, and I believe that none are ever formed.

Scarcely any trace of jacket cells can be seen in the group of archegonia of *fig. 16*, and they are never more than very feebly organized. In this respect the condition in Cephalotaxus as described by COKER (**9**) may be compared, where jacket cells are sometimes replaced by ordinary prothallial cells; and in Torreya where COULTER and LAND (**11**) note the absence of jacket cells until after fertilization. Their absence in this genus and in Widdringtonia is probably to be correlated with the small size of the archegonia.

If the nuclear phenomena in these partially abortive archegonia are to be taken as representing normal conditions, the central nucleus divides (*fig. 16*, sixth archegonium from bottom) to form the egg and ventral nuclei (see also the top archegonium). The ventral nucleus seems usually to disappear completely, leaving only a centrally placed egg nucleus, as shown in four of the archegonia in the figure.

The lower of the two proembryos referred to above (*fig. 16*) closely resembles the proembryo of Sequoia (LAWSON **20**), but the upper shows that more cells are formed than in that genus. It is noticeable that the proembryo practically fills the archegonium in each case. The only conifers previously described in which this is the case are Torreya (COULTER and LAND **11**) and Sequoia (LAWSON **20**). This fact is likely in all three cases to be correlated with the small size of the archegonium, and is probably of no phylogenetic importance. Only one of the several cells of the proembryo forms the suspensor and one forms the embryo. The others must disintegrate rapidly, as in the next stage they are no longer recognizable (*fig. 17*).

Figs. 16, *17*, and *21* show stages in the development of the multinucleate endosperm mentioned above. Although in early stages numerous uninucleate cells may be seen, as well as a large number of binucleate cells, yet the actual origin of the binucleate condition has only been indicated by two karyokinetic figures and the remains of a spindle between the two nuclei of one cell. Sometimes two nuclei come to lie almost in contact in a cell and have thus often suggested that direct division of the nucleus has occu red, but a careful search has failed to confirm this suggestion. It seems perfectly clear, therefore, that the binucleate (and in some cells multinucleate) condition arises by karyokinetic division of the original single nucleus.

Although in *Widdringtonia cupressoides* the evidence upon which this conclusion rests is perhaps slender, yet the same phenomenon has been seen in *W. Whytei* and in two species of Callitris. In the latter abundant evidence of the origin of the binucleate condition has been obtained in both *C. cupressiforme* and *C. Muelleri*.[3] Here a considerable number of nuclei have been found in every stage of karyokinetic division from earliest prophase to fully formed binucleate cells. The four-nucleate condition is very much less common in both Widdringtonia and Callitris, and it is therefore not surprising that a second mitotic division has not been met with, but doubtless it also occurs. A very limited number of cases is found in which five nuclei are present in a single cell.

The number of chromosomes in the division just mentioned is of course the reduced one. In Callitris both the haploid and diploid chromosomes have been approximately counted, the latter being about 24 and the former almost certainly 12 in both species mentioned. In *Widdringtonia cupressoides*, in one of the two nuclear figures mentioned above, the chromosomes were just starting to separate from the equatorial plane, and in the other they had advanced about half-way to the poles. It was possible to count the group of daughter chromosomes approximately in three out of the possible four cases, and the number was about 6 in each case. The sporophytic number has been found to be about 12 (certainly not more than 14). One dividing nucleus is figured (*fig. 22*) in which 12 chromosomes seem clearly indicated; this is from a very young embryo. It is curious in two genera so closely allied as Widdringtonia and Callitris that the number of chromosomes in the one should be approximately double that found in the other, but similar differences have been noted in even more closely related plants (GATES **15**, ROSENBERG **32, 33**). So far as I am aware, no case has previously been recorded in which a multinucleate prothallus persists in a conifer, but this is certainly the case in Widdringtonia, as *fig. 21* (from quite an old prothallus) clearly shows. An entirely binucleate prothallus is present in Cryptomeria at one stage (LAWSON **21**), but subsequent cell divisions

[3] I am not quite certain that the second species is correctly named, but both cones and foliage seem to agree very closely with description and figures given by MAIDEN (**24**).

reestablish the uninucleate condition. Multinucleate jacket cells are reported by LAWSON (**23**) in various Cupressineae, also by COULTER and LAND (**11**) in Torreya, and by KILDAHL (**18**) in Phyllocladus. Apparently no case has previously been reported, however, in which a multinucleate prothallus persists in the conifers, but the record may easily have been overlooked by the writer, owing to a large proportion of the literature not being available to him. LAWSON (**23**), however, makes no mention of such a case. Parallel cases are reported among the Gnetales (LAND **19**, PEARSON **28**), but this in itself is probably of little importance, especially as the origin of such cells is different in these cases.

The development of the embryo shows no peculiarities and closely resembles the general sequence of events as described for other conifers, an apical cell being organized for a very short time (*fig. 18*); the presence of embryonal tubes is to be noted in *fig. 19*. In the mature embryo the cotyledons are two (very rarely three) in number and usually of the same length; sometimes, however, one cotyledon is conspicuously shorter than the other.

COMPARISON WITH WIDDRINGTONIA WHYTEI AND CALLITRIS

One collection of *W. Whytei*, growing in the Tokai plantations, was made on January 11, 1909, but did not yield results of much importance. It clearly agrees with *W. cupressoides* in the presence of laterally placed archegonia and in the multinucleate endosperm.

Single collections were also made of three species of Callitris (*C. verrucosa*, *C. cupressiforme*, and *C. Muelleri*)[4] during the second week in January. Of four ovules of the first-named species sectioned, one showed secondary prothalli (*text fig. 1*), and in each the prothalli were in the free nuclear condition and one pollen tube only was found, which had penetrated a short way down the side of the prothallus and contained three nearly equal nuclei (*text fig. 3*). The upper of the three, however, stains more sharply than the other two and suggests the same sequence of events as described above for Widdringtonia. The ovules of the other two species contained young embryos in different stages of development (similar to *figs. 17–19*). The unfertilized archegonia are much too disorganized to make out any-

[4] See footnote, p. 171.

thing beyond the fact that they occur in a lateral group (or possibly more than one group). The formation of the multinucleate prothallial cells has already been described. Sufficient evidence has been obtained to indicate that the development in Callitris is essentially similar to that of Widdringtonia.

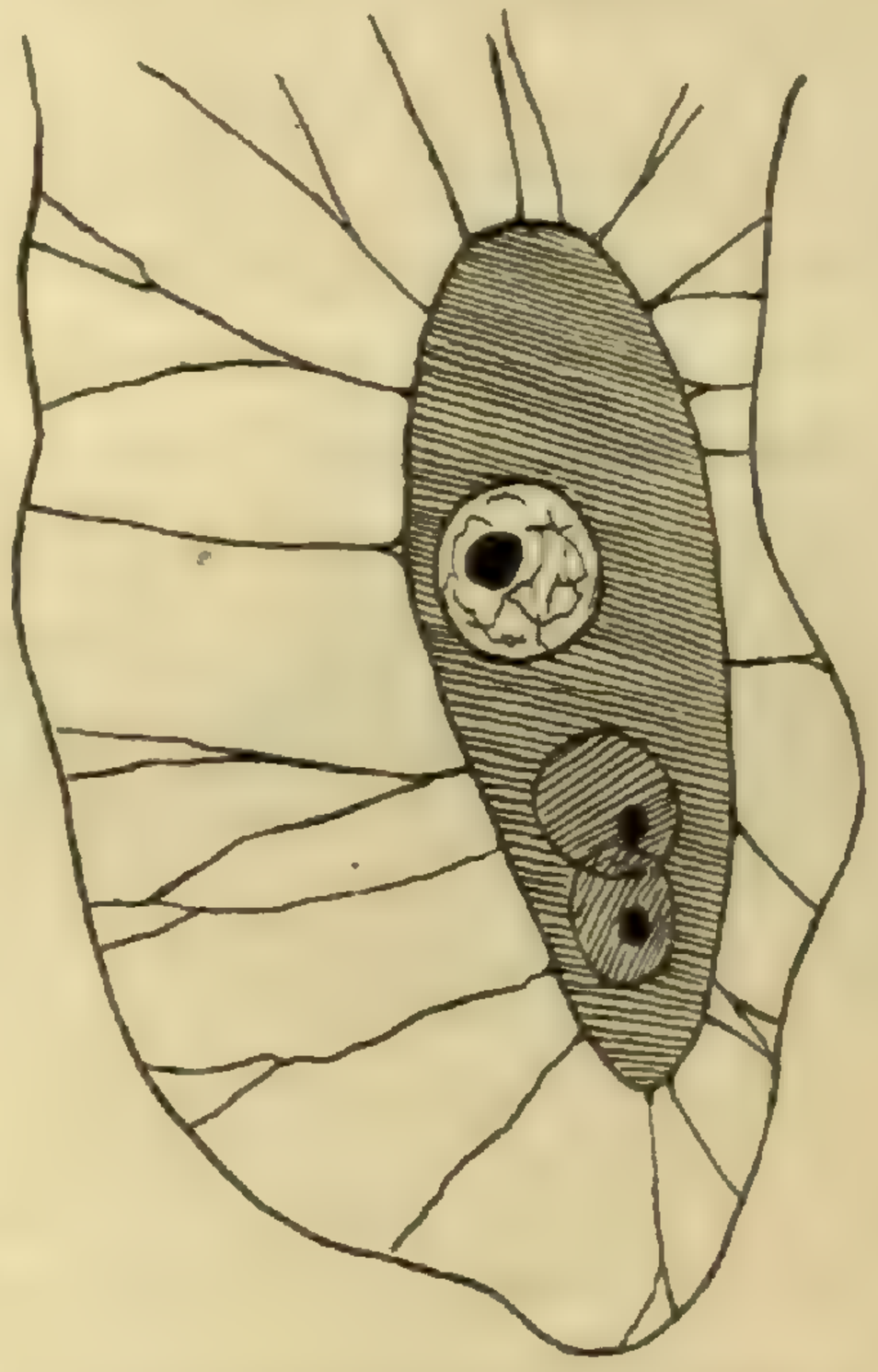

FIG. 3.—Tip of pollen tube of *Callitris verrucosa*. ×778.

DISCUSSION

With the exception of GOEBEL'S (**16**) figure of Tetraclinis (*Callitris quadrivalvis*) and COKER'S (**8**) statement about the pollen grain of Callitris, I am aware of no contribution to the knowledge of the gametophytes in the Actinostrobeae. As shown by MASTERS (**26**), there is a very close agreement between the four genera of this section of the Cupressineae in sporophyte characters, and the present investigation shows that the section is much more clearly differentiated from other Cupressineae in the gametophyte than in the sporophyte characters. Especially is this the case in the female gametophyte, where the peculiar position of the archegonia and the persistently multinucleate prothallus constitute a sharp distinction from typical Cupressineae. On the other hand, the position of the archegonia is similar to that found in Sequoia, and I hesitate to emphasize the differences in structure of the archegonia, as described above, until the development has been more closely followed. It has already been suggested that the Cupressineae have been derived from the Sequoiaceae, and it is quite possible that the Actinostrobeae constitute the connecting link between the two tribes.

It is just possible, however, that the developmental details may have a wider significance than this in indicating an approach to the conditions met with in the Gnetales. If the apparent absence of neck cells in the deep-seated archegonia is confirmed, a comparison is at once suggested with the multinucleate prothallial tubes of Tumboa

(Welwitschia).[5] A consideration of this question and also of the possible relation between the multinucleate prothallial cells of the two genera is better postponed, however, until PEARSON'S later researches on Tumboa have been published, and a closer series has been obtained in Widdringtonia.[6]

One interesting point, suggested by the fact that karyokinetic divisions occur in the prothallus at about the same time as the ventral nucleus is cut off, is that all the cells of the prothallus are potential archegonia. If the neck cells are really absent, the only essential difference between an archegonium with its egg and ventral nuclei and the other binucleate cells of the prothallus is one of size.

SUMMARY

1. The genus Widdringtonia is quite distinct from Callitris and should be kept as a distinct genus, excluding Tetraclinis.

2. A fixing agent not generally employed in cytological work has been found to give better results than chromacetic mixtures with and without osmic acid.

3. The male gametophyte is of the most reduced type yet recorded in the gymnosperms, no division of the microspore occurring until some time after pollination, and the tube and stalk nuclei disappearing before the body cell divides and before the archegonium initials can be recognized.

4. A very large number of megaspores are probably formed, but only one of these ever forms a prothallus.

5. The early development of the prothallus is perfectly normal.

6. A very large number of archegonia (over 50) are formed, and these are arranged in a number of groups near the margin of the prothallus on the side down which the pollen tube grows. They are confined to the upper half of the prothallus (but absent from the apex).

7. The archegonia arise from deep-seated cells of the prothallus and never grow through to the margin.

[5] See RENDLE'S remarks (**30**) on the correct generic name of this remarkable plant.

[6] Since the above was written, an abstract of PEARSON'S paper (**29**) has been published. He now finds the origin of the endosperm in Tumboa to be quite different from what was expected (cf. PEARSON **28**).

8. Jacket cells are either absent or sometimes very feebly developed, and apparently neck cells are entirely absent from the archegonia.

9. The central nucleus of the archegonium probably divides to form the egg nucleus and a ventral nucleus.

10. By karyokinetic divisions in the prothallus cells, about and after the time of fertilization, they become binucleate and in some cases four- or even five-nucleate.

11. The multinucleate condition persists, differing in this respect from Cryptomeria, as well as in the origin of the binucleate cells.

12. The haploid and diploid numbers of chromosomes are respectively 6 and 12 (approximately). In two species of Callitris the numbers are approximately 12 and 24.

13. From the occurrence of proembryos and embryos in pairs it is concluded that fertilization of two archegonia is effected by two sperms from a single pollen tube.

14. The proembryo contains eight or more cells, one of which forms a suspensor and the other the embryo. Early stages of the proembryo are wanting.

15. Embryo development is quite normal, and embryonal tubes are formed.

16. A resemblance is noted in certain points with the development of *Sequoia sempervirens*.

17. A comparison is provisionally suggested with the Gnetales, especially with the genus Tumboa.

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35. *SOKOLOWA, MLLE. C., Naissance de l'endosperme dans le sac embryonnaire de quelques gymnospermes. Bull. Soc. Imp. Nat. Moscou. 1890 (1891).

EXPLANATION OF PLATE XI

All figures were drawn with the aid of a Zeiss microscope, lenses, and camera lucida; *figs. 4, 8, 11, 14,* and *22* with a 2^{mm} oil-immersion objective , and the other figures with various dry objectives. Magnifications were measured directly by comparison with a stage micrometer in each case. Each figure is oriented with the long axis of cone or ovule vertical except *figs. 8* and *22*. The sections were cut out with a Cambridge rocking microtome to a thickness of 4 to 10 μ.

FIG. 1.—Median longitudinal section of very young ovule, not yet pollinated; January 7, 1909. ×180.

FIG. 2.—Median longitudinal section of staminate cone, slightly diagrammatic; only the apical three-fourths of the cone are shown; January 7, 1909. ×36.

FIG. 3.—Median longitudinal section of a pollinated ovule (drawn from two sections); January 7, 1909. ×147.

FIG. 4.—Germinating pollen grain from a similar ovule to that shown in *fig. 3;* January 7, 1909. ×725.

FIG. 5.—Median longitudinal section of central part of ovule, showing very large number of megaspores (?); May 3, 1908. ×180.

FIG. 6.—Sketch of whole ovule in optical section (cleared in cedar); oil at the apex of the nucellus is a pollen tube; June 30, 1908. ×10.

FIG. 7.—Pollen tube in apex of nucellus of similar age to that shown in *fig. 6;* May 25, 1908. ×180.

FIG. 8.—Very small part of prothallus of same ovule as *fig. 7;* vacuole to the right, membrane to the left. ×1720.

FIG. 9.—Small part of a prothallus in the process of forming cell walls; August 25, 1908. ×310.

FIG. 10.—The tip of a pollen tube which has penetrated nearly half-way down the prothallus of *fig. 9* (cut obliquely and drawn from two sections). ×310.

FIG. 11.—The body cell of *fig. 10* more highly magnified. ×725.

FIG. 12.—Upper half of median longitudinal section of nucellus after wall formation is complete in the prothallus, showing the characteristic position of the pollen tube; August 25, 1908. ×24.

FIG. 13.—Tip of pollen tube of *fig. 12*. ×310.

FIG. 14.—Body cell nucleus of *fig. 13*. ×1240.

FIG. 15.—Upper half of median longitudinal section of nucellus, *n*, and prothallus, *p*, showing position of pollen tube and of archegonia; drawn from several sections; *t*, pollen tube; January 7, 1909. ×28.

FIG. 16.—The lowest group of archegonia of *fig. 15*, two containing proembryos. ×130.

FIG. 17.—Suspensor bearing a single embryo cell at its apex; note multinucleate prothallus cells in this and previous figure; *s*, suspensor; *e*, embryo cell; January 7, 1909. ×310.

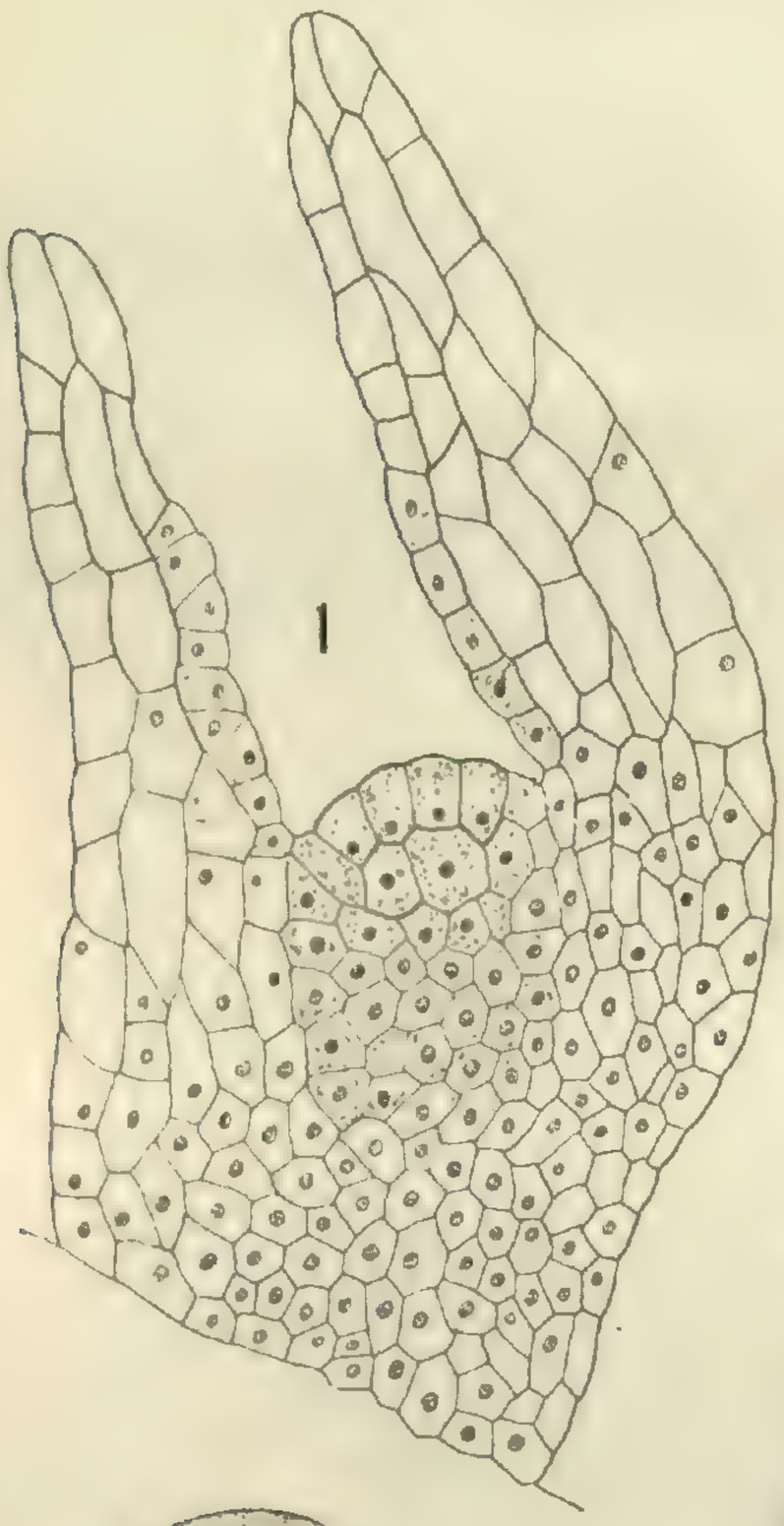
FIG. 18.—Very young embryo in median longitudinal section; March 8, 1908. ×505.

FIG. 19.—Older embryo, showing dermatogen differentiated and embryonal tubes; January 7, 1909. ×490.

FIG. 20.—Outline sketch of median longitudinal section of nearly mature embryo; March 8, 1908. ×9.

FIG. 21.—Two multinucleate cells from an old prothallus; March 8, 1908. ×540.

FIG. 22.—Dividing nucleus from an embryo between the ages of those shown in *figs. 17* and *18* respectively. ×1500.



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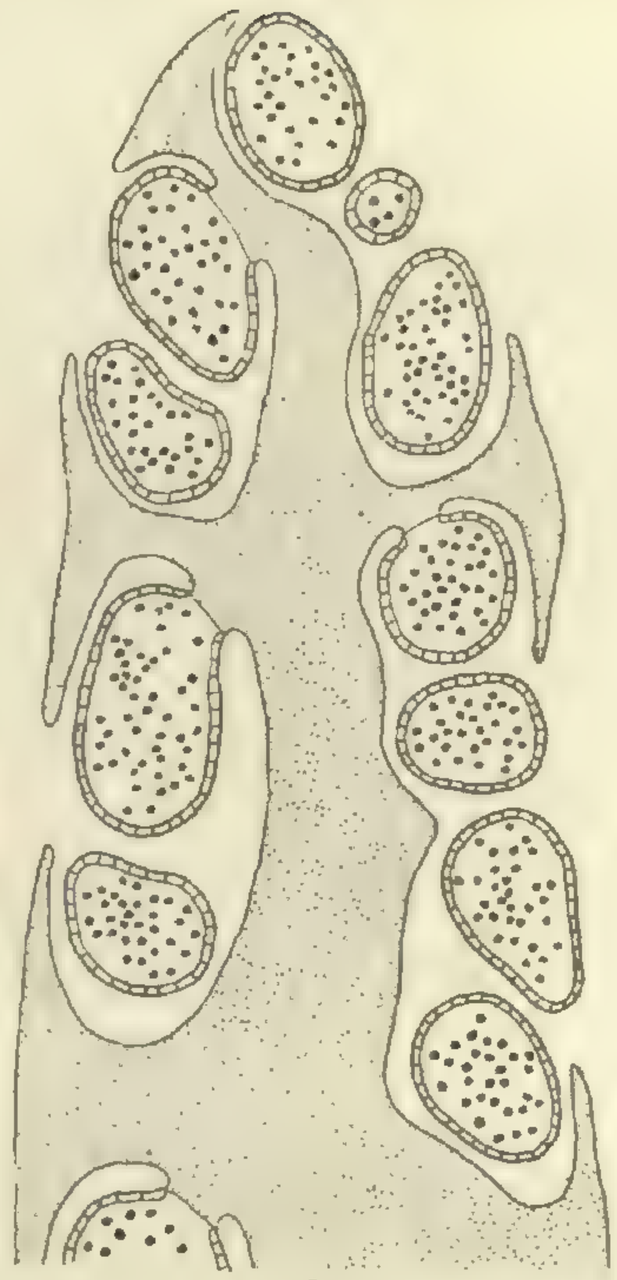

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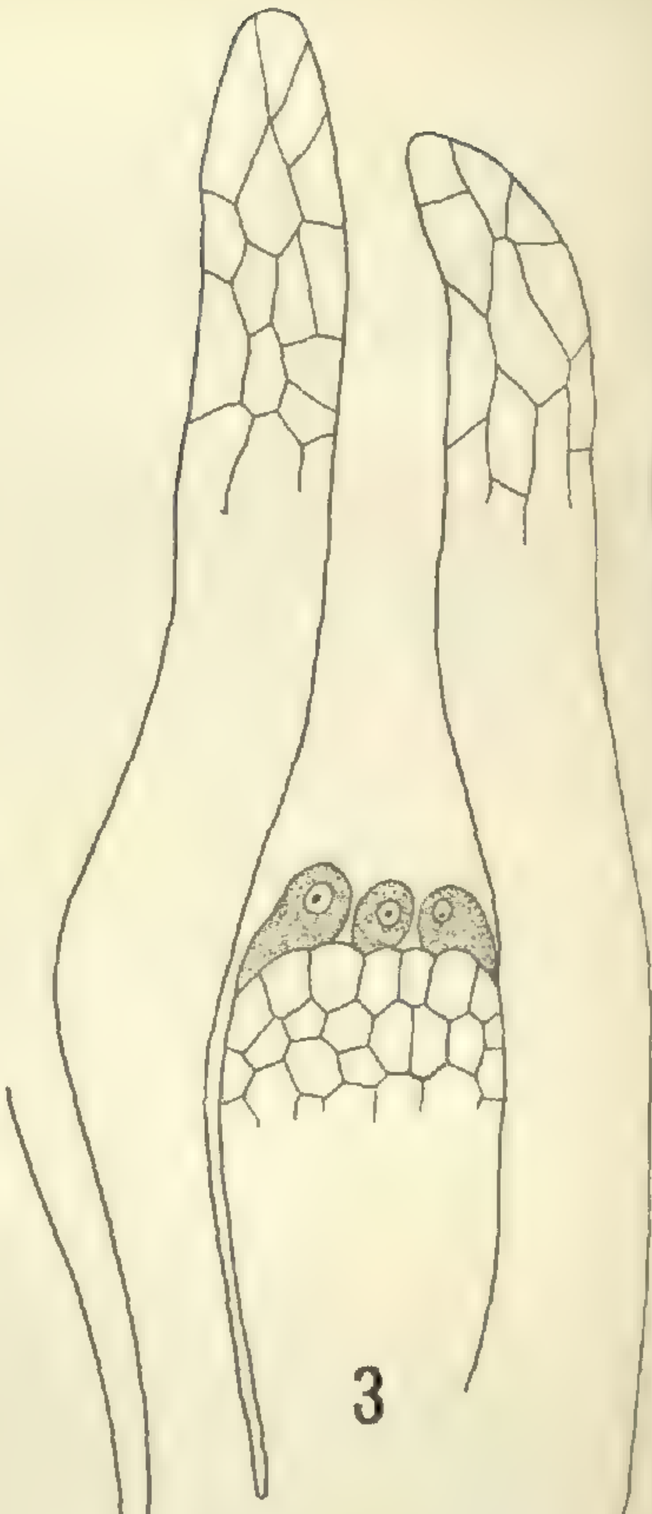

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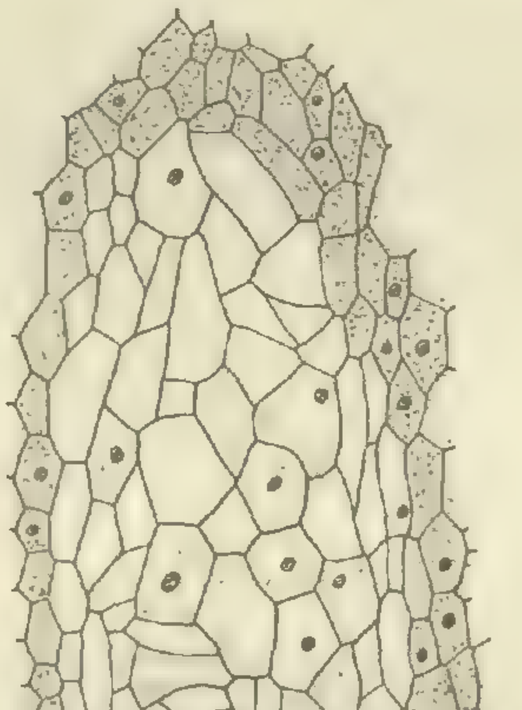

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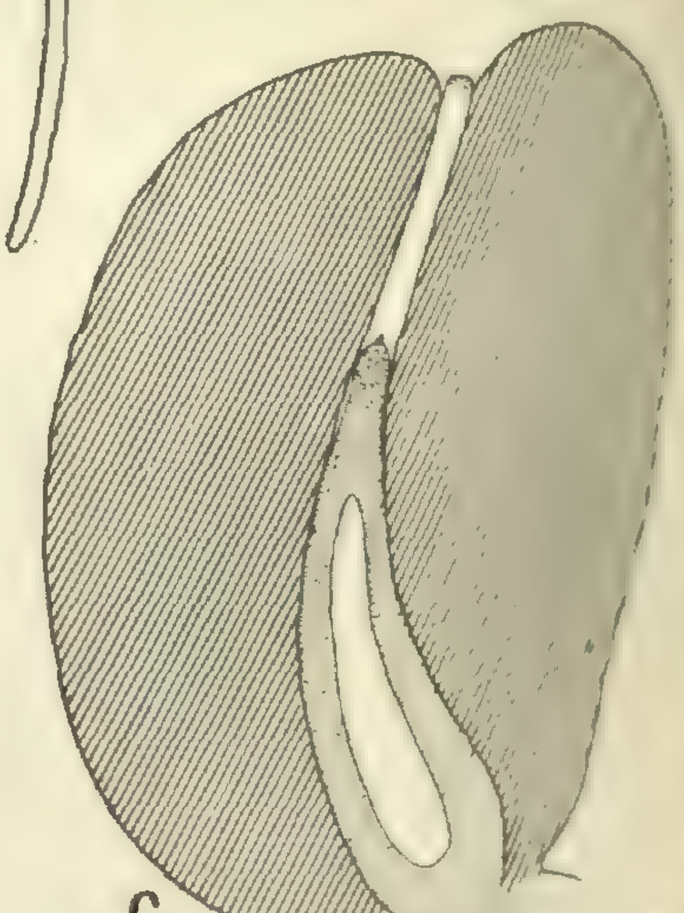

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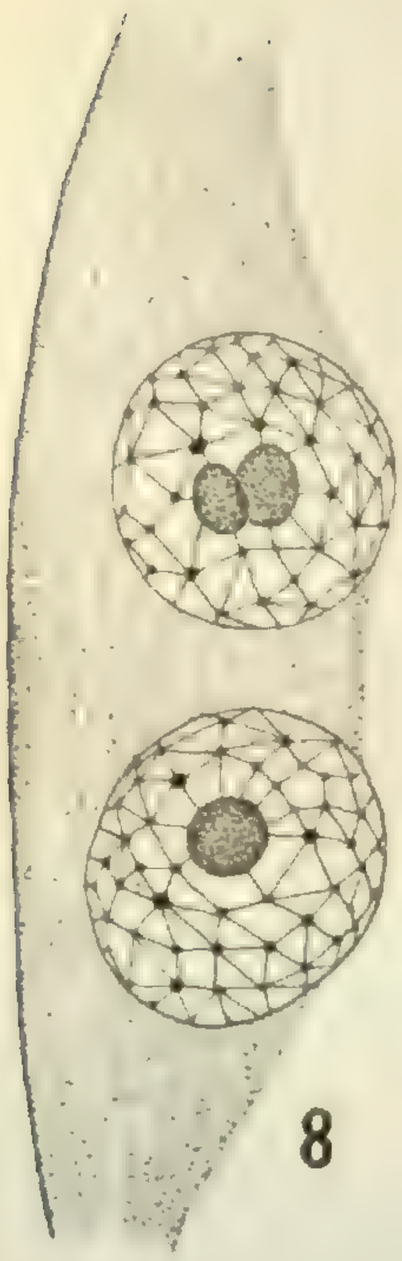

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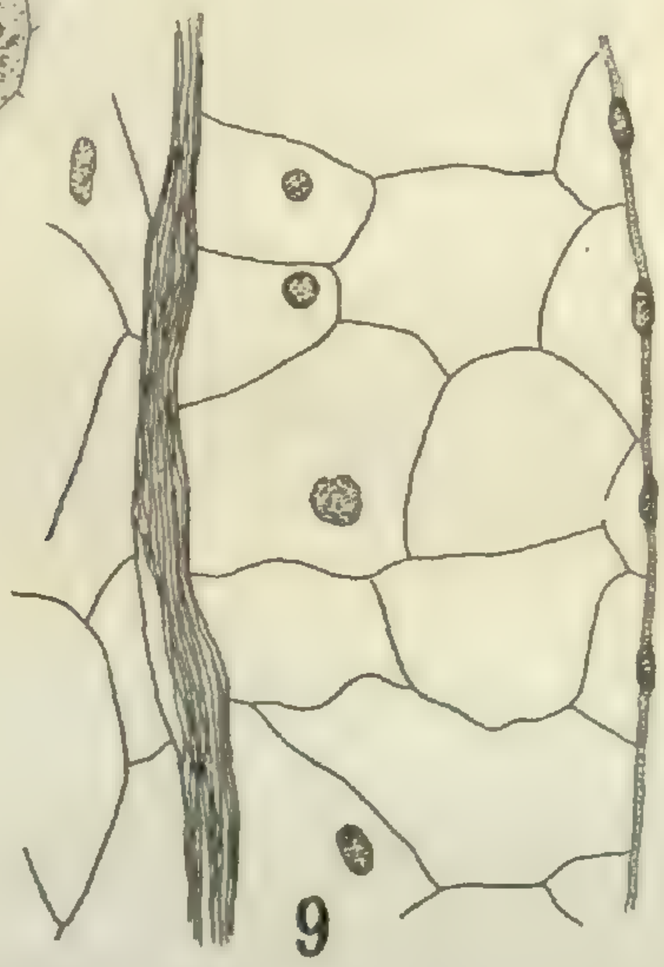

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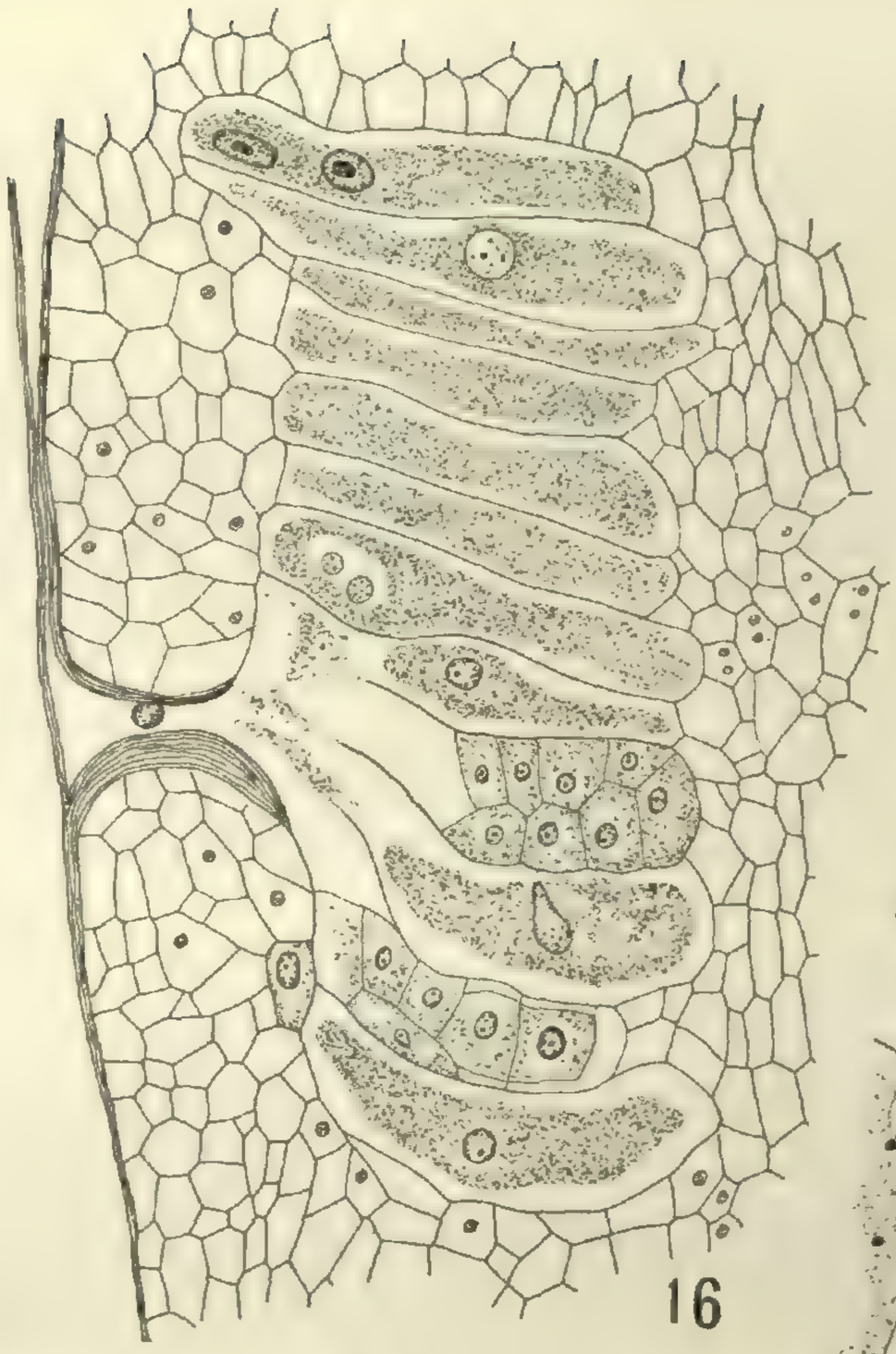

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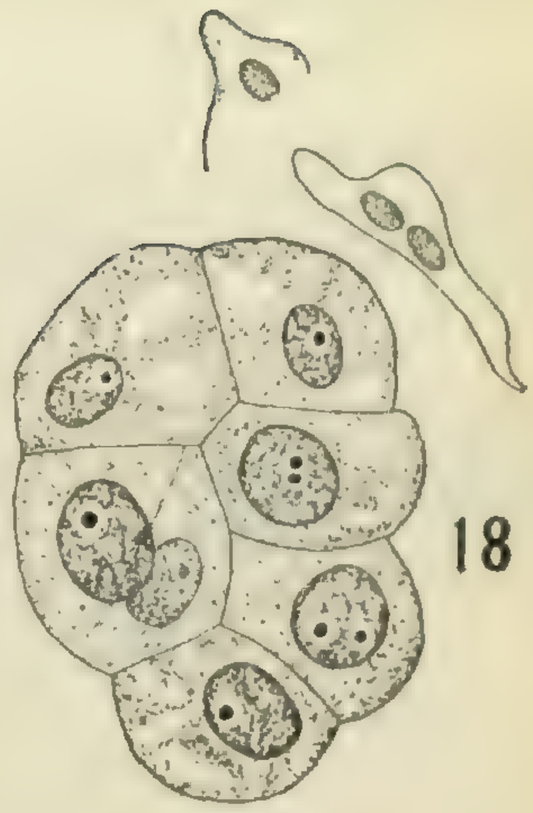

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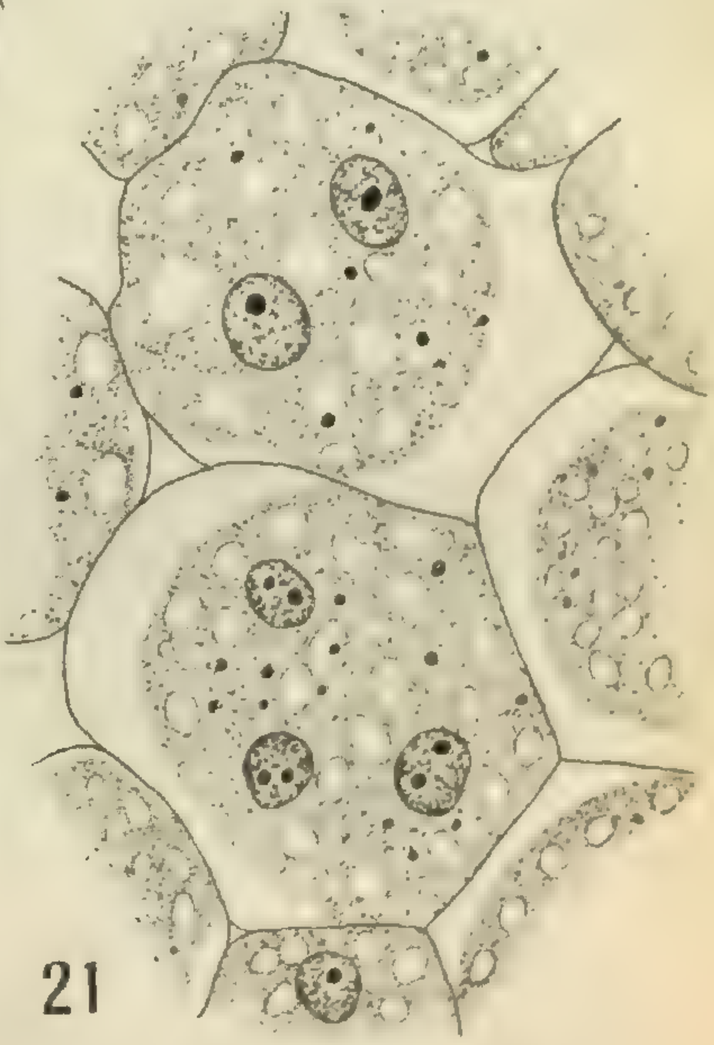

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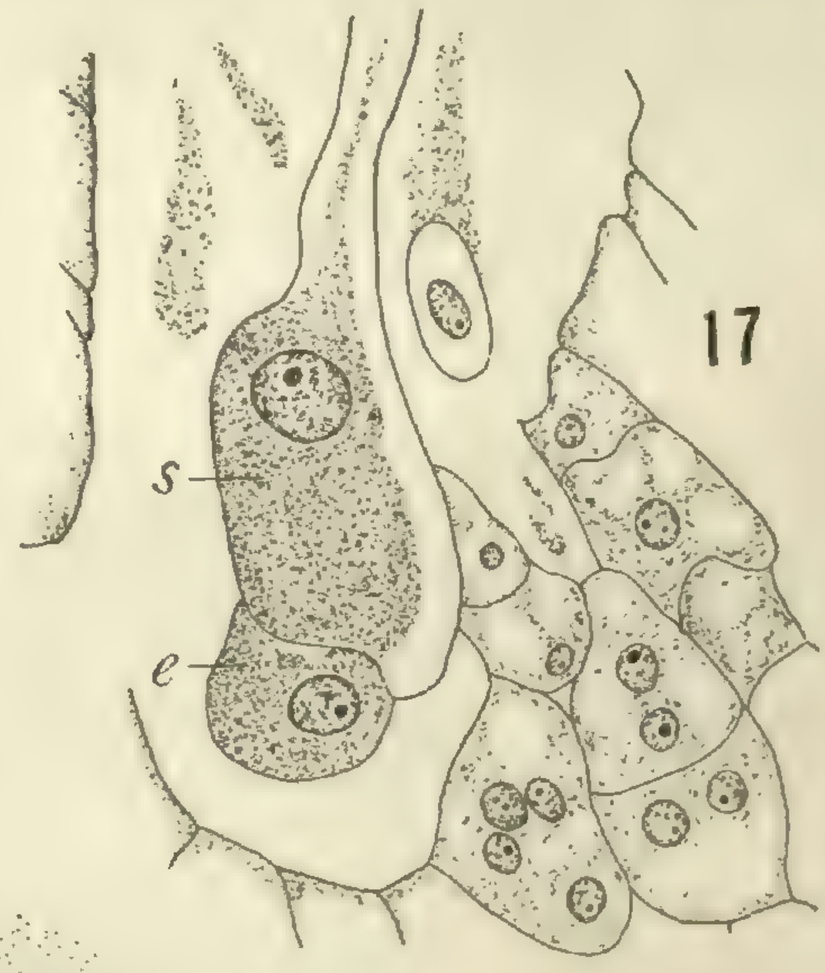

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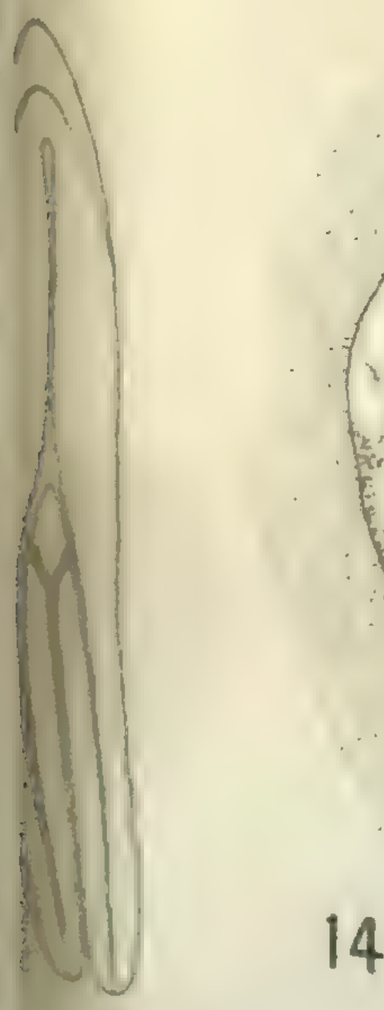

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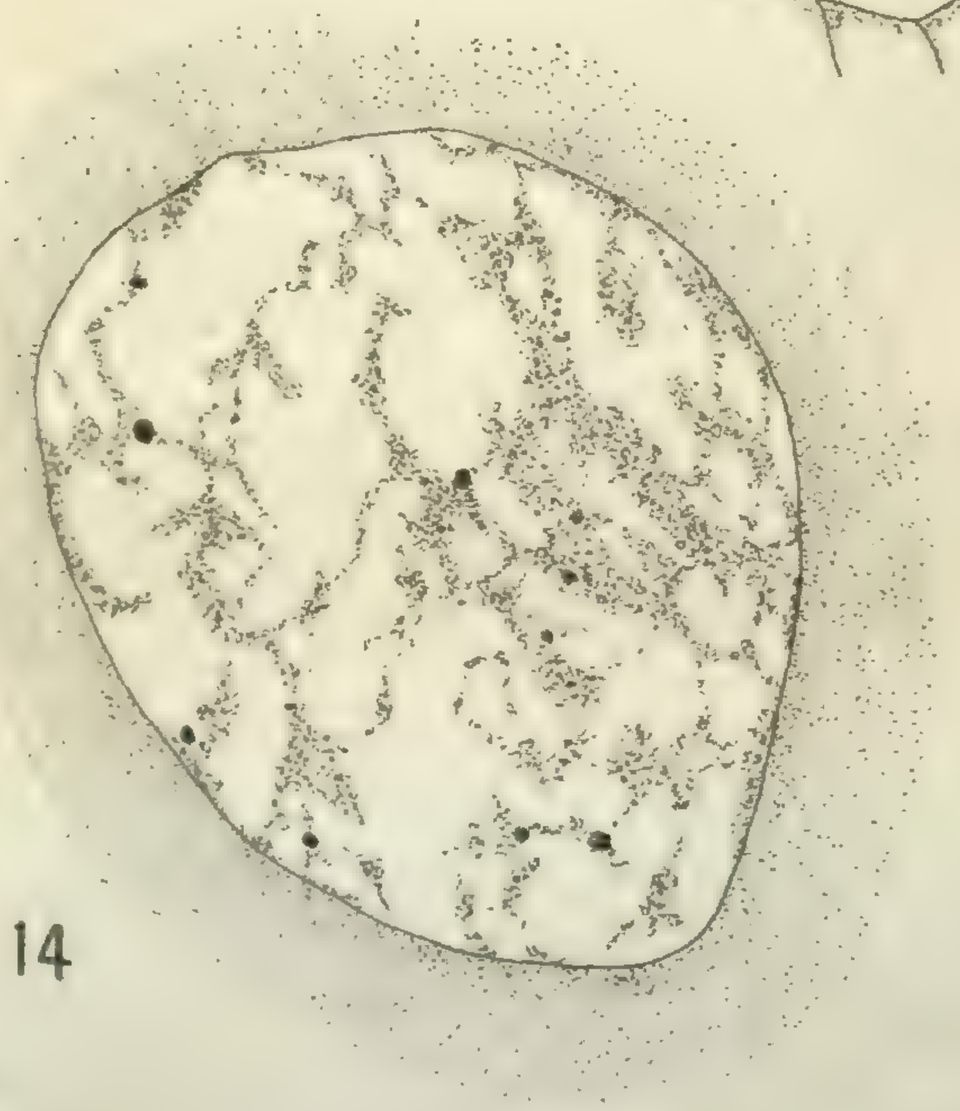

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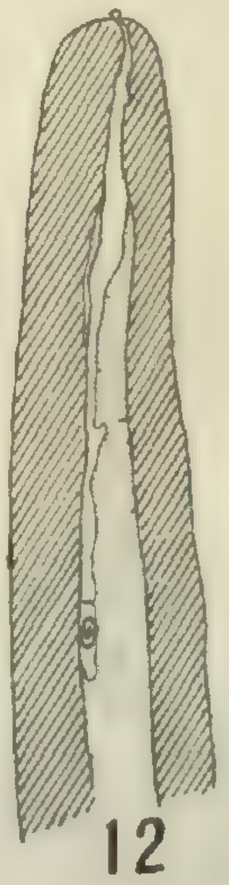

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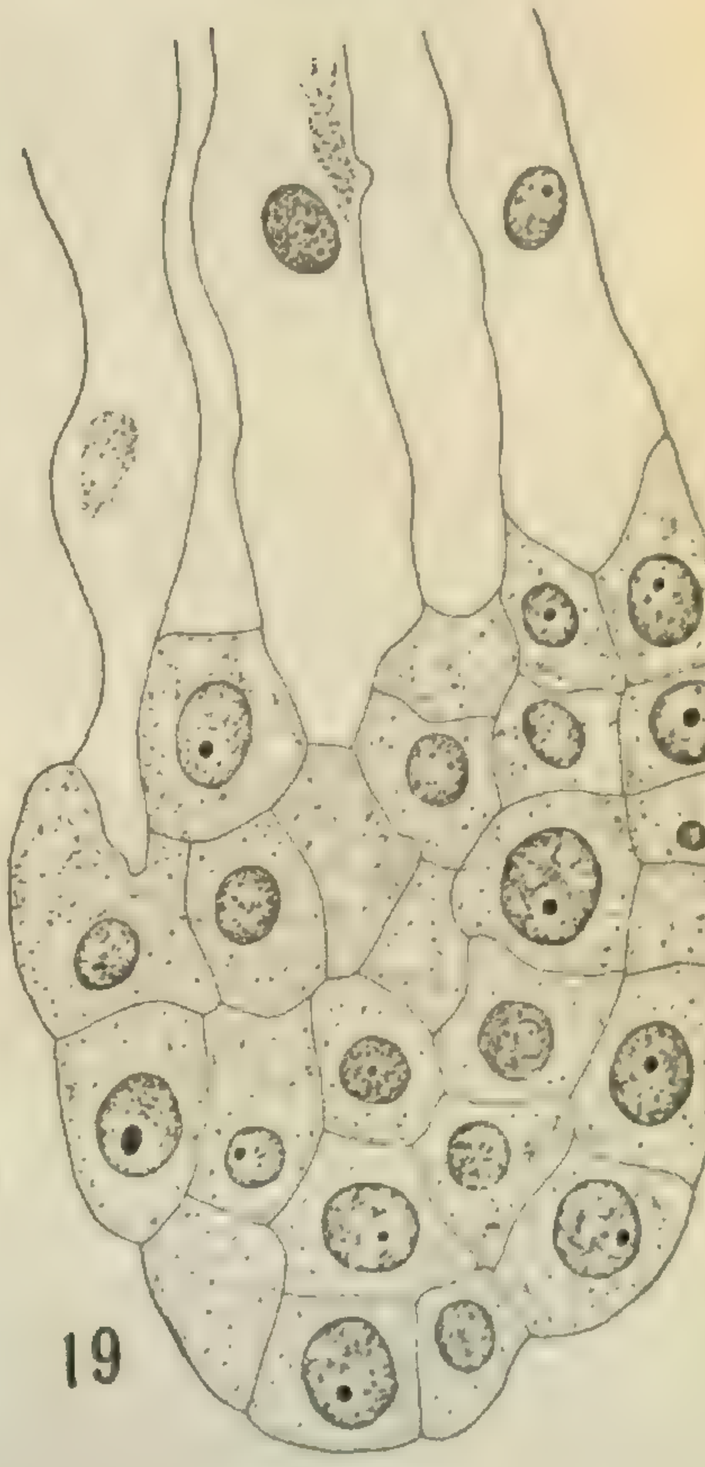

19

THE BEHAVIOR OF THE CHROMOSOMES IN *OENOTHERA LATA*×*O. GIGAS*

OCNTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 128

REGINALD RUGGLES GATES

(WITH PLATES XII–XIV)

The hybrid which forms the subject of this paper is of peculiar interest because one of its parents has double the number of chromosomes possessed by the other, *O. lata* having usually 14 chromosomes and *O. gigas* 28. Very few cases of this sort are known, either in plants or animals. But a further complication arises in the fact that *O. gigas* is known to have originated from *O. Lamarckiana*, which also has 14 chromosomes, and *O. gigas* has in all probability attained the tetraploid number by a duplication of the chromosome set present in *O. Lamarckiana*.[1] Then if fertilization took place in the ordinary manner, the hybrid *O. lata*×*O. gigas* would be expected to have 21 chromosomes, 7 derived from the *lata* egg and 14 (which is probably a double set of *Lamarckiana* chromosomes) derived from the male cell of *gigas*. Under these circumstances, the behavior of the chromosomes in the hybrid, especially during the period of reduction and germ-cell formation, is a matter of especial interest.

The general results regarding chromosome numbers and distribution were obtained some time ago, and a brief statement was published (10), but the cytological evidence is here presented for the

[1] I have discussed this matter in another paper to appear in the *Archiv für Zellforschung*. DEVRIES in a footnote to a recent paper (5) considers it an important question whether the doubling in *O. gigas* is accomplished through a longitudinal or a transverse splitting. In all the cases, of which I am aware, of chromosome multiplication under experimental conditions without nuclear division (BOVERI 4, LILLIE 23), the chromosomes evidently divide longitudinally as in ordinary somatic mitoses so that it seems probable that the division in *O. gigas* has also been longitudinal (on the very probable assumption that it occurred after fertilization). This view is further supported by the fact that the chromosomes of *O. gigas* show no apparent change in size or shape from those of *O. Lamarckiana*. If the division had been transverse, such a change might perhaps be expected. It is not impossible that in *Drosera longifolia*, which has 40 small chromosomes (ROSENBERG 31), while *D. rotundifolia* has 20 larger ones, there has been a transverse split of the *D. rotundifolia* chromosomes.

first time. Many of the drawings for these figures were completed about two years ago, but my interest in other phases of this work has postponed their publication until now. The earlier stages of reduction in Oenothera, up to the end of the heterotypic mitosis, have already been described in detail (**11**), so that this paper will deal chiefly with the later stages, beginning with the metaphase of the heterotypic mitosis.

The plants from which these studies were made were grown at Wood's Hole, Mass., in 1905 and 1906, from seeds of DEVRIES. The results show that in some cases the number of chromosomes is undoubtedly 21, while in one individual it was 20.[2] The number is undoubtedly constant in an individual, however, as shown by a large number of counts, which demonstrated constantly 20 in one case and 21 in the other.

The external characters were not studied with sufficient care at that time to describe them accurately, but from my notes they appear to have been intermediate between *O. lata* and *O. gigas*. DEVRIES has described in a recent paper (**5**) the hybrids of *O. gigas* with other forms. I have called attention elsewhere to the fact that the behavior of *O. gigas* in hybridization, as well as its number of chromosomes, places it in a different category from the other mutants of *O. Lamarckiana*. DEVRIES finds that *O. gigas*×*O. Lamarckiana* forms a constant race intermediate between the parents, at least to the second hybrid generation. *O. gigas*×*O. Lamarckiana*, *O. Lamarckiana* ×*O. gigas*, *O. gigas*×*O. brevistylis*, *O. gigas*×*O. rubrinervis*, and *O. rubrinervis*×*O. gigas*, all give constant hybrid races which are externally alike in all these crosses. *O. lata*×*O. gigas*, however, gives in the F_1 two types, about 50 per cent. of each; type I intermediate between *O. lata* and *O. gigas*, type II intermediate between *O. Lamarckiana* and *O. gigas*. These presumably would all have about 21 chromosomes. There were 133 plants in the culture of this hybrid in 1907 and a smaller number in 1908.

Miss LUTZ (**24**) from a study of 40 individuals of *O. lata*×*O. gigas* finds more complex conditions in this cross, though apparently she

[2] In my first paper (**8**) this individual was thought to be *O. lata*×*O. Lamarckiana*, but was afterward found to be derived from fertilization by foreign *gigas* pollen, this particular seed package not having been guarded as was supposed when the seeds were planted.

has failed clearly to recognize the types included in her class III. She divides the offspring into three classes. Class I has the characters of pure *O. lata*, and the two individuals which appeared are each said to have 15 chromosomes.[3] Class II consists of *O. gigas* plants having about 30 chromosomes. There were six of these plants, and their presence cannot be accounted for by the ordinary methods of fertilization. If the *O. gigas* male cell united with two *O. lata* nuclei in the embryo sac, this would account for the origin of the *O. gigas* number of chromosomes. But GEERTS (**12, 13**) finds that the embryo sac of Oenothera contains only four nuclei, the egg, two synergids, and one polar nucleus, so that the possibilities here are more limited than in an 8-nucleate sac.[4] The class III of Miss LUTZ apparently includes both the types of DEVRIES'S cross, but they are not characterized so that a comparison can be made.

These interesting facts all show that there is still much to be explained regarding *O. gigas* and its hybrids. In the paper already referred to, I have shown that, in all the tissues examined, the cells are larger in *O. gigas* than in *O. Lamarckiana*, though the percentage of increase varies in different tissues. The production of the tetraploid number of chromosomes results in larger nuclei and larger cells, and this in turn in many cases produces larger organs. Certain changes which are found in the relative dimensions of the cells will account for the altered shape of certain organs. Thus the differences between *O. Lamarckiana* and *O. gigas* can probably be analyzed into two factors, (1) increased size of the cells, and (2) altered relative dimensions of the cells in certain cases. The former undoubtedly

[3] The presence of pure *O. lata* in this cross strongly suggests apogamy in this mutant, and it seems not unlikely that a condition exists resembling in part that found by ROSENBERG (**32**) in *Hieracium excellens*, in which part of the embryo sacs are developed after normal reduction processes and require fertilization, while occasionally embryo sacs are produced without reduction (apogamously or aposporously), in which case the egg develops without fertilization. I hope to test this hypothesis experimentally this summer.

[4] Whether an individual with the tetraploid number of chromosomes would have the *O. gigas* characters, owing to its *number* of chromosomes rather than their *source*, is a question I have considered in a forthcoming paper (footnote 1). The view that the number is the main factor is supported, perhaps, by the fact that all the mutants of *O. Lamarckiana* experimented with (except *O. lata* which is peculiar in its crosses), when crossed with *O. gigas* give a constant hybrid of the same type in every case.

results directly from the doubling of the chromosome number; at least the double number of chromosomes and the larger size of cells occur simultaneously. Whether the latter is a necessary consequence upon the increase in cell size and a resulting change in cell relations, or is due to an independent factor, is uncertain. It should be said that characters, such as leaf shape, which might be accounted for by a change in the relative dimensions of the cells (though I have not as yet made measurements of the leaf cells to determine this), are extremely variable, and it seems not unlikely that this extreme variability may result from variation in cell dimensions consequent upon the readjustment to the double chromosome number.

DESCRIPTION

The cytological account will begin with the telophase of the heterotypic mitosis in the pollen mother cell. The ten figures in plate XII are from the plant having 20 chromosomes, and were drawn on a smaller scale than those of the other two plates. At this stage the chromosomes can be counted with perfect accuracy. A large number of counts of this telophase show that 10 chromosomes enter each daughter nucleus. In a number of cases 10 were counted at each end of the same spindle. In several instances 11 were found in one of the daughter groups, and in a few cases it was possible to show that the corresponding daughter group contained only 9 chromosomes. In this plant, then, there are 20 chromosomes which segregate into two groups of 10 each in the reduction division, one chromosome occasionally going to the wrong group. Counts of somatic cells, made long ago in tissues of the anther, also showed that 20 chromosomes were present.

The material from which the figures in plate XII were drawn was subjected to an exceptionally high temperature in the process of imbedding, and in a few cases this has apparently affected the shape of the viscous chromosomes. *Figs.* 1–5 are early telophases before the formation of a nuclear membrane. In *fig.* 1 the chromosomes nearly all show their bivalent nature, and in most of them the two halves are dumb-bell shaped. This clubbing of the chromosomes at the ends is a common phenomenon both at this time and in the telophase of the homotypic and somatic mitoses. I have already

referred to these appearances in an earlier paper (**9,** p. 19). In *fig. 2* the chromosomes are more irregular in shape, and their bivalent character is not so evident. There are 11 chromosomes in the daughter group, and examination of the adjacent sections showed that there were 9 and no more at the other end of the spindle. The globular black bodies seen in the figure are frequently found scattered near the periphery of the cytoplasm. They stain like chromatin with Haidenhain's iron alum hematoxylin, but their chemical nature is unknown. *Figs. 3–5* each show 10 chromosomes. *Fig. 6* is a telophase with 11 chromosomes, the next section showing 9 at the other end of the spindle. *Fig. 1* is an exact polar view, the other figures being more or less oblique cuts of the spindle, but the spindle fibers are not represented. *Figs. 7, 8* are somewhat later stages in side view, soon after the nuclear membrane is formed. Ten chromosomes are present in each. In *figs. 9, 10* are shown in outline the loop-shaped chromosomes of two somatic cells. Each has 20 chromosomes. The cells are from the middle layers of the anther wall.

Plates XIII and XIV deal with plants having 21 chromosomes. *Fig. 11* is a side view of the heterotypic spindle, showing 20 or 21 chromosomes. It is usually difficult to count the chromosomes exactly at this time in a side view of the spindle, on account of the close aggregation of some of them, and the presence of spindle fibers. The chromosomes are almost never regularly oriented in an equatorial plate on the heterotypic spindle. An examination of the literature shows that in most plants and animals a flat equatorial plate is formed in the metaphase of this mitosis as in other mitoses, although a few forms constitute exceptions. But the homotypic mitosis in Oenothera has always a very definite equatorial plate, in which the chromosomes are oriented in a single plane. There can be little doubt that the irregularities in chromosome distribution arise from this failure of the chromosomes to be regularly paired and oriented on the heterotypic spindle. The irregularities in distribution certainly arise at this time.

I have recently confirmed practically all the events of reduction by a study of the wild *O. biennis*, so that this account of reduction applies to the genus Oenothera in general and is not the result of mutative conditions. This will be referred to again later.

Fig. 12 is another cell in telophase. One nucleus is uncut, showing 10 bivalent chromosomes. The other was sectioned by the knife. *Fig. 13* shows 11 chromosomes and a number of very small nucleoli. Usually only one or two larger nucleoli are present. In *fig. 14* there are 12 bivalent chromosomes. In such cases 9 appear at the opposite end of the spindle. *Figs. 17, 18* are very early telophases just after the nuclear membrane has been formed. The daughter nuclei grow very quickly to the size of *figs. 19, 20*, each of which shows 11 bivalent chromosomes, while in the nucleus represented in *fig. 21* there are only 9. In general I have found that the nucleus having 9 chromosomes is likely to be appreciably smaller than one having 11. Evidently the amount of karyolymph secreted by a nucleus is a function of its number of chromosomes. This is also shown by the many cases of single chromosomes left behind in the cytoplasm and forming small nuclei during reduction, as observed in various forms, especially in hybrids.

These drawings of telophases are nearly all from nuclei which are uncut by the knife in sectioning. The sections are usually 10 μ thick, so that in a majority of cases the nucleus is completely contained in one section. The nuclear membrane is conspicuous; hence by focusing it can be determined with certainty that the membrane is uncut and that the contents of the nucleus are intact. All the nuclei in which counts have been made have been first shown to be intact in this manner. Moreover, whenever the chromosomes in both daughter nuclei could be counted, their sum was invariably found to be 21, whether 10+11 or, as occurred in a few cases, 9+12.

There are a number of interesting features about the telophase, which I have studied with particular care. In the first place it is in the best stage for counting the chromosomes with absolute accuracy, and is even better than diakinesis, because of the reduced number of chromosomes. In my last paper on reduction (**11**) I showed that pairing before the reduction division only takes place to a limited extent, and that the shapes characteristic of heterotypic chromosomes are therefore usually absent at this time, as will be seen from *figs. 26–34* of the paper referred to. During the period of interkinesis, however, the entire chromosomes of the heterotypic mitosis having split to form bivalents in which the two parts are closely held together,

show the characteristic X, Y, V, H, K shapes, etc. (See *figs. 12–14, 19–21.*)

A large number of counts of the chromosomes were made in these telophases, and it was found that the numbers 10 and 11 occurred with approximately equal frequency, while the numbers 9 and 12 were only occasional. Every single case which was admitted as a count could be determined with absolute certainty as a case in which there were, for instance, just 10 chromosomes, no more and no less.

In the anaphase of the heterotypic mitosis, the chromosomes as they pass to the poles are nearly globular or somewhat elongated in shape, and are at first closely massed at the poles of the spindle. There is considerable variation in the time of appearance of the split in these chromosomes, though they usually appear bivalent in the early telophase. They appear to come into actual contact at this time, forming a compact group, but never fusing or uniting. They very soon begin to separate, however, and as they do so nuclear sap appears between and to a lesser extent around them. Then the nuclear membrane appears where the karyolymph comes in contact with the cytoplasm. The nucleus so formed is at first very small, but grows rapidly to its full size by the increase in nuclear sap.

LAWSON (**20**) has described this process in detail in *Passiflora coerulea*. He found that the chromosomes fused into a single mass in the telophase, and that the karyolymph begins to be secreted within this chromatic mass and later comes to surround it. The cytoplasm, coming in contact with the karyolymph, forms the nuclear membrane, which is therefore the limiting membrane of the cytoplasm, just as is the tonoplast of a vacuole. It is undoubtedly true that the nuclear membrane is formed where the karyolymph comes in contact with the cytoplasm, although it must remain uncertain whether the karyolymph is secreted by the chromosomes or merely attracted and accumulated about them from the cytoplasm. There must be extremely little if any cytoplasm included in the nucleus so formed, because the nuclear membrane appears close about the chromosome group when the chromosomes are still almost in contact. This supports GRÉGOIRE'S observations (**14, 15**), that the structural contents of the resting nucleus are formed wholly from the chromosomes, and there can be no doubt, in this case at least, that the

daughter nucleus is formed entirely from the chromosomes and the karyolymph in which they float. This is to me a strong observational evidence of chromosome continuity.

Unlike LAWSON's description for *Passiflora coerulea*, the chromosomes in Oenothera certainly do not fuse in the telophase of the heterotypic mitosis, but maintain their separate identity completely at first, and usually more or less completely thoughout the period of interkinesis. Occasionally stages are found which indicate that they string out and anastomose to some extent during a late stage of interkinesis, partly losing their sharp boundaries, but this stage apparently does not very often occur.

Nucleoli may be formed *de novo* in these daughter nuclei, as I have described previously (**8,** p. 93). The chromosomes in this telophase are all clearly bivalents, in which the halves are closely in contact. I have examined thousands of nuclei in this stage and have never seen the halves other than more or less closely in contact. On the other hand, it is universally true in my observations that no two chromosome bivalents are ever found in contact. Not only is this the case, but they are invariably distributed at approximately equal distances from each other, just within the nuclear membrane. I have never seen an exception to this in any Oenothera studied. The position of the chromosomes might be explained by supposing that they are attached to the nuclear membrane from the first, and are thus carried outward as the nucleus grows.[5] In many cases the chromosomes appear actually to be attached to the nuclear membrane, or at least to be lying very closely against it. This, however, leaves unexplained the fact that the chromosomes are never in contact with each other at this time, and further that they are very generally placed at approximately equal distances from each other, just within the nuclear wall. All these facts point to the supposition that the

[5] It might also be supposed that in the dehydration processes preparatory to mounting, the chromosomes would be drawn against the nuclear membrane. But in such a case one would undoubtedly find the chromosomes occasionally massed on one side of the nucleus or irregularly placed, instead of being always at regular intervals about the periphery. From the regularity of their placing I have no doubt that the chromosomes occupy their original positions within the nucleus, and there is no indication that they are ever disturbed by the processes of fixation, imbedding, and staining, when properly carried out.

chromosome bivalents are mutually repelled. It is true that in the early telophase the chromosomes form a close group, so that they certainly cannot be repelled at that time, but may be attracted. However, the medium in which bodies float frequently changes their qualities of attraction and repulsion, and it appears that the repulsion first develops after the appearance of the karyolymph in which the chromosomes float. The facts all suggest that the chromosome bivalents mutually repel each other at this time, while the halves of these are held together, probably by attraction.

The studies of WILSON (**37**) and others on insect chromosomes show that there are *selective* attractions between certain chromosomes at the time of synapsis or at some other period of meiosis. These of course require something more specific than electromagnetic forces to explain them.

Fig. 22 shows one of the nuclei of a pollen mother cell in the prophase of the homotypic mitosis. Eleven chromosomes are present, having the same bivalent structure as in the telophase of the previous mitosis. Both nuclei always go through the various stages of the second mitosis simultaneously. The method of spindle formation has not been studied with great care, but corresponds with the Gladiolus type of multipolar spindle formation (LAWSON **19, 21**). There is no indication of an intra-nuclear network, the spindle being wholly extra-nuclear in origin. A portion of the weft of fibers surrounding the nuclear membrane remains *in situ* when the latter disappears, forming a close meshwork, against which the chromosomes lie. The fibers then rearrange themselves; the fibrillae from the cones already formed come in and become attached to the chromosomes; finally the spindle becomes bipolar by the rearrangement of the fibers forming the cones.

The numerous papers on spindle formation in angiosperms need not be cited here. Among the more recent critical studies of this structure may be mentioned that of BERGHS (**2**), who insists that the distinction between kinoplasm and trophoplasm will not hold; that the spindle results simply from the gradual orientation of the material of the cytoplasmic reticulum, and returns to a reticulum after mitosis.

Sometimes spindle formation begins on one side of the nucleus, as in *fig. 23*. In such cases the cones of the multipolar spindle may

be formed and the nuclear membrane may have disappeared on one side of the nucleus before any indication of spindle formation has appeared on the other side.

Fig. 15 is a polar view of the two homotypic spindles in metaphase. There are 11 chromosomes on one spindle and 10 on the other. *Fig. 25* is another at the same stage, showing 10 chromosomes. In *fig. 24* the spindles are at right angles and all the chromosomes are not shown. In the side view of the spindle some of the chromosomes appear like tetrads, owing to their bivalent structure. On the homotypic spindle, before the chromosomes divide, they are very regularly oriented in a single plane in the equatorial plate, as in *figs. 15, 25*. This contrasts strikingly with the heterotypic spindle, in which the chromosomes are scattered for a considerable distance along the long axis of the spindle, so that there is usually no metaphase, strictly speaking.

Fig. 26 is an early anaphase of the homotypic mitosis, showing one spindle in side view and one in polar view. In the polar view 20 chromosomes are found by focusing through a short distance, and the remaining 2 are found on the next section. In the side view the chromosomes could not all be counted, but presumably there were 20 after division. Two pale-staining nucleoli still persist in the cytoplasm.

Fig. 16 shows three of the nuclei in the telophase of the homotypic mitosis. Many of the chromosomes have a characteristic two-lobed or dumb-bell shape. One chromosome is left behind in the cytoplasm, leaving 10 chromosomes each in two of the daughter nuclei. This two-lobed shape is a characteristic appearance of the chromosomes in the telophase of somatic mitoses, but presumably bears no relation to the next mitosis, because if this were the beginning of a split for the next mitosis it would indicate that the division of the chromosomes is transverse. But metaphase and anaphase stages of somatic mitoses show that the chromosomes divide longitudinally. Of course the possibility that the transverse axis of a chromosome in telophase should regularly become its longitudinal axis before the next metaphase, is not excluded, although this seems unlikely.

The great difference in the size of the figures made it impossible to arrange them in order on the plates. The magnification is the same

as in my last paper in this journal (11), so that the figures can be directly compared.

DISCUSSION

The history of any ontogeny is the history of the transformation of chemical metabolism into definite structures, or rather the eventuation of a series of chemical processes in the production of a series of physical structures. Morphologists and cytologists map the succession of structures appearing and call it a series of events. They are not unmindful, however, that the primary process is the metabolism, the structures its by-products, which in turn, so far as they are capable of continuing metabolism, produce other structures and, particularly in the adult organism, structures like themselves. Similarly, the chromosomes of the germ nuclei must be thought of as definite aggregations of chemical materials, which initiate or take part in certain forms of metabolism. The chromosomes themselves do not (at any rate not wholly) control their growth or division, but this is more or less subject to the conditions of temperature, etc., in which they are placed, as the work of ERDMANN (7) and others has shown. Similarly, as the complex of physical and chemical conditions in the nucleus and cell undergoes changes, the chromosomes change from the compact to the alveolate or distributed condition, or *vice versa*, etc. The cytologists who record these events are aware that chemical transformations are continually going on, and that the changes in visible physical structures are the external concomitant of such chemical processes.

Chemical reactions in the test tube, however complex, do not lead to the production of any structures more complex than crystals or flocculent masses of various sorts. There are indications that living matter frequently has the properties of liquid crystals, but why do the infinitely more complex reactions of living matter build structures of such relative permanency and of tremendous intricacy? The problem of individual development, from this standpoint, is the question, How do certain forms of chemical metabolism result in the production of certain visible physical structures or characters? Of course, our present microscopic appliances do not permit us to know how many structural steps, if any, there may be between the interacting molecular masses and the finest structures visible under our

highest powers, but it is by no means necessary to assume that there are such. This digression will indicate to some extent, perhaps, the viewpoint of the writer in connection with the cytological aspects of this work.

No attempt will be made to discuss here the literature of reduction, only a very few of the recent papers being referred to. The discussion of matters of cytological detail will be taken up at another time.

One important matter, which was discussed in a former paper (**11**), concerns the method of chromosome reduction, i. e., whether there is a pairing of threads about the time of synizesis and whether the spirem afterward breaks into a single series or two parallel series of chromosomes. In the paper referred to I established the fact (as will be conceded, I think, after a study of *figs. 20–32*, particularly, of that paper) that the spirem breaks into a single and not a double chain of chromosomes. The possibility of finding a series of stages which really demonstrates this depends on the shape of the chromosomes in Oenothera. They are relatively short and thick, like many animal chromosomes, and quite unlike the twisted and tangled chromosomes of such forms as Lilium, whose relationships are so difficult to interpret. It is a curious fact that so many of the critical studies on reduction in plants have been made on forms with long narrow chromosomes, although many of the interpretations of critical stages can be made with much greater ease and certainty on forms having short and stout chromosomes.

In the paper just referred to, I concluded that the method was probably different in different genera, there being a side-by-side pairing of threads in some forms (parasynapsis),[6] but an end-to-end arrangement of the chromosomes to form a single spirem (telosynapsis)[6] in other forms. Subsequent papers by various investigators continue to describe both these methods, and the evidence in certain cases is so clear that I think there can remain no doubt that both these general methods occur in plants. MONTGOMERY concluded in 1898 (**26**) that there are different types of reduction in animals.

In a recent study of Fucus, YAMANOUCHI (**39**) shows clearly that at the time of reduction the ragged nuclear reticulum is gradually transformed into a single continuous thread, which enters into

[6] These convenient terms follow the usage of WILSON (**36**).

synapsis. This thread then becomes rearranged into regular loops converging to one side of the nucleus and forming the "bouquet" stage of EISEN (**6**). This stage is characteristic of many animals, in which it has been described by JANSSENS and DUMEZ (**17**), STEVENS (**34, 35**), MARÉCHAL (**25**), and JORDAN (**18**), to mention only a few. Each loop is composed of two chromosomes arranged end to end. Similarly the spirem in Fucus is made up of the maternal and paternal chromosomes arranged endwise in a single thread. This is what I have shown to be the case in Oenothera (**11**), although Oenothera has no typical "bouquet" stage, nor has any other angiosperm, so far as I am aware, though the second contraction phase, characteristic of various forms, probably corresponds to it. OVERTON (**30**) states that this stage does not occur in the plants with short chromosomes which he has examined, yet it occurs in Oenothera, in which the definitive chromosomes are very short and frequently almost globular. The second contraction phase is a well-marked stage of meiosis in Oenothera.

I may mention a few of the recent accounts involving an end-to-end arrangement of the chromosomes to form the spirem (telosynapsis). MOTTIER finds (**29**) that in Podophyllum, Lilium, and Tradescantia the two members of each bivalent chromosome were not side-by-side in the spirem, representing the halves of the longitudinally split thread, but were arranged end-to-end in the chromatic thread. LEWIS (**22**), in a study of Pinus and Thuja, finds no pairing of threads, but cross-segmentation of a single post-synaptic spirem to form the chromosomes. Just what relation his *fig. 15*, which indicates a reticulum as occurring about the time of chromosome formation, bears to the other stages, it is hard to say. LEWIS ventures the opinion that the second division is probably qualitative, but with a manifest lack of evidence to support it. SCHAFFNER (**33**), in Agave, finds bodies which he believes are bivalent prochromosomes, and a single spirem which segments to form the twelve bivalent chromosomes.

Among very recent accounts of reduction which involve a longitudinal pairing of threads (parasynapsis) may be mentioned that of GRÉGOIRE (**16**), with figures involving the critical stages in Lilium, Osmunda, and Allium; YAMANOUCHI (**38**) in Nephrodium; OVER-

TON (**30**) in Thalictrum, Calycanthus, and Richardia. ALLEN'S extensive earlier paper on Lilium (**1**) should also be mentioned. Although, in such cases as Lilium, both accounts of reduction are given for the same form by different authors, yet the evidence from such forms as Nephrodium on the one hand, and Tradescantia, Oenothera, and Fucus on the other hand, makes it very difficult to deny that both methods occur. A comparison of a wide range of forms whose reduction phenomena show many differences, will doubtless lead finally to an explanation of the nature and meaning of these differences. It is very evident that the time has passed when all the accounts of reduction in plants can be brought under a single scheme. The task of the future will be to interpret the meaning of the differences observed in various forms. Are they matters merely of cell mechanics, or are they related to hereditary processes? It is not impossible that correlations will be found between the method of reduction in an organism and its type of hereditary behavior. In other words, the phenomena of reduction and the distribution of elements during meiosis may condition to some extent the hereditary behavior of a plant or animal. This is already known to be the case with sex in insects. But it does not seem worth while entering into such theoretical possibilities on the basis of our present knowledge.

The fact that in this Oenothera hybrid the number of chromosomes in the pollen mother cells is the sum of those which enter the fusion nucleus at the time of fertilization, is to me clear evidence that each chromosome in the germ cells is the genetic descendant of one of the chromosomes in the fertilized egg. The work of various investigators seems to have established the genetic continuity of chromosomes from generation to generation of individuals. The manner in which the chromosomes are distributed during reduction in this Oenothera hybrid clearly shows that they behave as individuals at this time. WILSON'S recent account of the supernumerary chromosomes in Metapodius (**37**), a genus of Hemiptera, shows certain points of resemblance to the condition in Oenothera. WILSON finds that certain chromosomes (the idiochromosomes) may be present in duplicate, or may even have several representatives in the cells of certain individuals, which nevertheless show no external differences, the number of chromosomes being fixed for any individual, but varying

in different individuals from 21 to 28 according to the number of supernumerary chromosomes present. The distribution of the supernumeraries during spermatogenesis is also irregular, so that the number varies in the different sperm nuclei of an individual. Occasional irregularities in the distribution of idiochromosomes during reduction were observed in the form having 22 chromosomes, both idiochromosomes passing to the same cell. This is believed to be the origin of the variation in different individuals, the supernumeraries being merely duplicates of the idiochromosomes.

Similarly, I have observed occasional irregularities in the chromosome distribution during reduction in nearly all the mutants of Oenothera examined, and this doubtless accounts for the different numbers of chromosomes found in different individuals, the extra chromosomes being duplicates of others already present, and the presence of 20 chromosomes in one individual of *O. lata*×*O. gigas* being due to the absence of one chromosome from one of the germ cells which produced that individual.

MONTGOMERY (**27**) first proposed the theory that in synapsis homologous chromosomes of maternal and paternal origin pair with each other, and much subsequent work, especially with animal chromosomes, sustains that view. As I have already shown, pairing of Oenothera chromosomes frequently fails to take place, and this allows a chance for the irregularities in chromosome distribution which occur. BOVERI (**3**, p. 54) has suggested that when there is an absence of pairing in synapsis in any organism, its chromosomes are hereditarily equivalent. In Oenothera there is clearly a tendency to pair, but it is only partly carried out. I have already compared (**11**, p. 25) the behavior of the chromosomes in this hybrid with the condition described by ROSENBERG (**31**) in the Drosera hybrid having 10+20 chromosomes. The method of segregation of chromosomes in the heterotypic mitosis is evidently different in the two hybrids, and this was used as an argument in favor of a different method of reduction in the two cases. In the Oenothera hybrid the 10–11 segregation shows that the segregation cannot be between chromosomes of maternal and paternal origin, but it must be remembered in this connection that the 14 paternal chromosomes are very probably a double set of 7 *O. Lamarckiana* chromosomes. The regu-

larity with which the 10–11 segregation takes place, indicates that it is not merely a matter of chance, but that some mechanism, perhaps connected with the spindle, determines this regularity. A study of later generations of this hybrid should throw much light on the question whether the chromosomes of Oenothera are really unlike.

MORGAN (**28**) has recently shown that in certain phylloxerans, in which a generation of winged individuals produces (parthenogenetically) sexual males and females, the eggs are of two kinds, the female-producing eggs being large and the male-producing eggs small. Further, the females thus produced have the same number of chromosomes as the parthenogenetic females, while the males have two chromosomes less. Thus, in the formation of the polar body of the male egg, two extra chromosomes are extruded, so that the somatic cells of the male contain two less chromosomes than those of the female. Evidently there is here some sex-determining factor which antedates the chromosomal differences with the two kinds of eggs, and yet the chromosomes are the instruments of this factor, for the extrusion of the two chromosomes always precedes the development of the male individual. Studies of this sort will doubtless give us clearer notions regarding the respective rôles of chromosomes and cytoplasm in heredity.

In a former paper (**11**) I suggested that if the chromosomes of Oenothera are unlike in their hereditary capacities, then the occasional irregularities I have described in the chromosome distributions on the heterotypic spindle would furnish a possible basis for the appearance of a series of types, such as the mutants of *O. Lamarckiana*. I have since studied the reduction phenomena in *O. biennis* and other types, an account of which will be published later. *O. biennis*, in at least some of its geographical races, appears to be stable under ordinary conditions, though MACDOUGAL (**24a**) has obtained atypical forms by injections into its ovaries. The loose and frequently unpaired arrangement of the chromosomes in the central region of the heterotypic spindle is evidently a delicately adjusted condition which can easily be thrown out of balance. Yet in some way the balance is ordinarily maintained and is only occasionally deranged in such a way that a different chromosome distribution results. It is not impossible that the condition of mutation in *O. Lamarckiana*

has arisen through a disturbance by some means of this delicate condition of balance.

But it is useless to speculate on such possibilities until it is known whether the chromosomes of Oenothera are really unlike, and at present there is no evidence in favor of this view except the inferential evidence from chromosomes in general. The fact that chromosomes reappear with each mitosis, showing the same differences where visible differences exist (except in the cases of amitosis, whose status in relation to hereditary processes is not at present understood), would seem to favor the assumption that they maintain their identity and are unlike. But I need not cite here the general arguments in favor of this hypothesis. The more definite evidence, such as that of the sex chromosomes in insects, is not necessarily of universal application.

SUMMARY

1. *O. lata*×*O. gigas* has 21 chromosomes in its somatic cells, 7 of maternal origin (*O. lata*) and 14 of paternal origin (*O. gigas*). In one individual the number was 20, owing probably to the absence of one chromosome from one of the germ cells which produced this individual.

2. These chromosomes segregate at the time of reduction, so that in individuals having 21 chromosomes half the germ cells receive 10 and half 11 chromosomes. In the individual having 20 chromosomes, 10 enter each germ cell. Occasionally one chromosome goes to the wrong pole of the spindle, so that in plants having 21 chromosomes a few germ cells are found having 9 or 12 chromosomes and in the plant with 20 chromosomes, occasional germ cells have 9 or 11 chromosomes. This irregularity in chromosome distribution accounts for the fact that different individuals in a race in some cases have different numbers of chromosomes.

3. The 10–11 segregation of chromosomes in the formation of the germ cells of this hybrid shows that there is not here a pairing and separation of homologous chromosomes of maternal and paternal origin, but that the segregation tends to be into two numerically equal groups.

4. Evidence from this and other work shows that there are two general methods of chromosome reduction in plants, one involving

a side-by-side pairing of chromatin threads (parasynapsis) to form a double spirem; the other involving an end-to-end arrangement (telosynapsis) of the maternal and paternal chromosomes to form a single spirem, which may afterward split longitudinally.

5. The behavior of the chromosomes in Oenothera supports the view of their genetic continuity from generation to generation, the number present in any individual being always the sum of the chromosomes in the germ cells from which that individual was formed.

6. If the chromosomes of Oenothera are unlike in their hereditary capacities their behavior furnishes a not improbable basis for the phenomena of mutation in *Oenothera Lamarckiana*. But it remains to be proven that the chromosomes of Oenothera are of unequal hereditary value.

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EXPLANATION OF PLATES XII–XIV

The figures were drawn with the aid of a Bausch & Lomb camera and a Zeiss apochromatic objective 2^{mm}, ap. 1.30, particular care being taken to represent the chromosomes accurately. The figures in plate XII were drawn with a Zeiss compensating ocular 12, those of plates XIII and XIV with ocular 18. All are reduced one-fourth in reproduction, which leaves the figures in plates XIII and XIV magnified as in my last paper (**11**) on reduction.

PLATE XII

All figures from the plant having 20 chromosomes.

FIG. 1.—Early telophase of the heterotypic mitosis in the pollen mother cells before formation of the nuclear membrane; 10 chromosomes, all clearly bivalent; in many the halves are dumb-bell shape.

FIG. 2.—Same stage; 11 chromosomes, many irregular; 9 chromosomes at the opposite end of this spindle; dark staining bodies in periphery of cytoplasm.

FIG. 3.—Telophase, showing 10 chromosomes of various shapes.

FIG. 4.—Telophase, showing 10 chromosomes, most of them clearly bivalents.

FIG. 5.—Same as *fig. 4.*

FIG. 6.—Telophase, showing 11 chromosomes, with no indication of their bivalent character; 9 at opposite end of spindle; apparent size of chromosomes in all figures varies to a certain extent according to the depth of stain.

FIGS. 7, 8.—Later telophases, soon after formation of nuclear membrane, each showing 10 chromosomes.

FIGS. 9, 10.—Metaphase groups of chromosomes in somatic cells from middle layers of anther wall, each showing 20 chromosomes; indications of an arrangement of the chromosomes in pairs.

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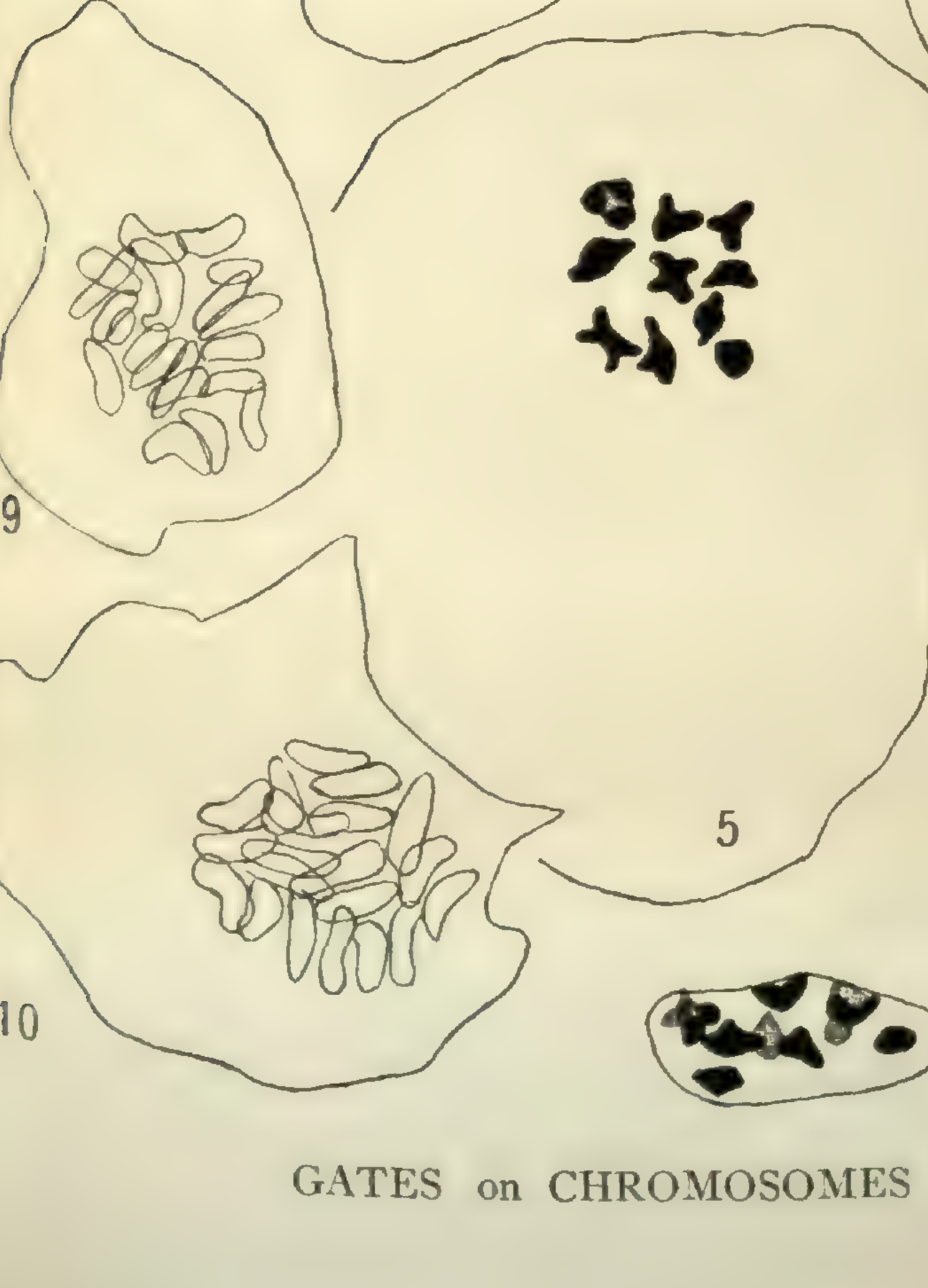

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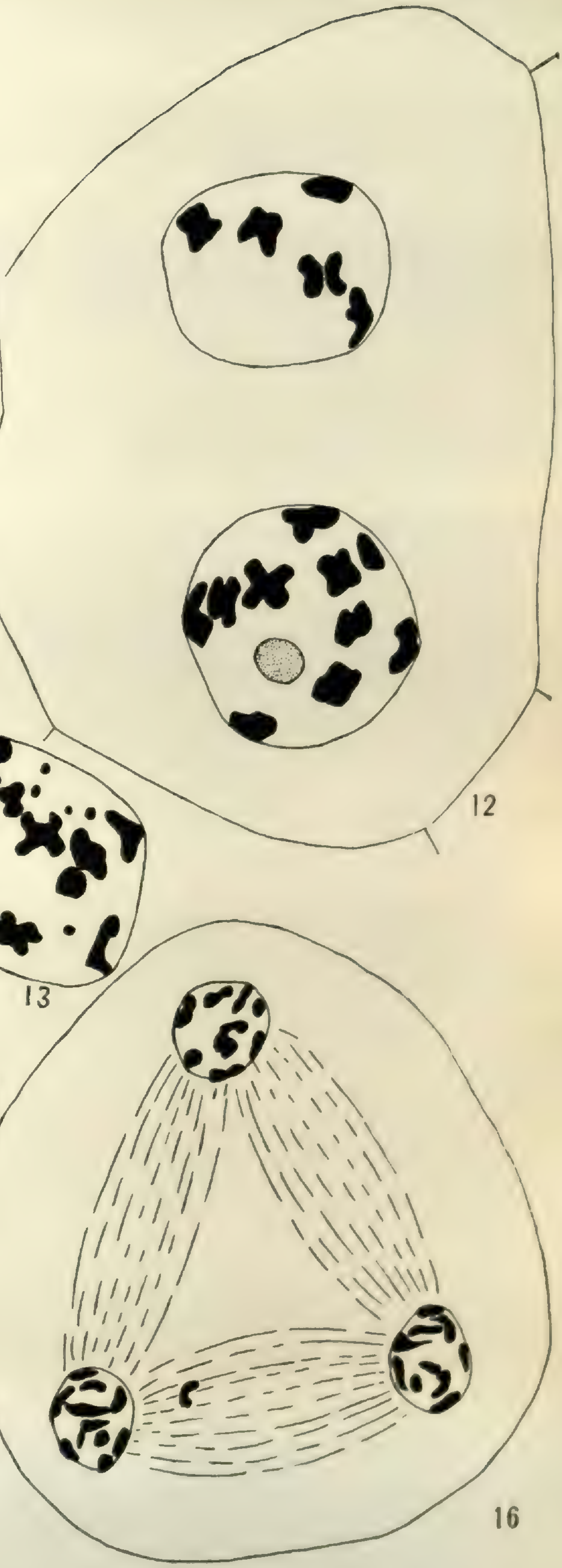
GATES on CHROMOSOMES of OENOTHERA



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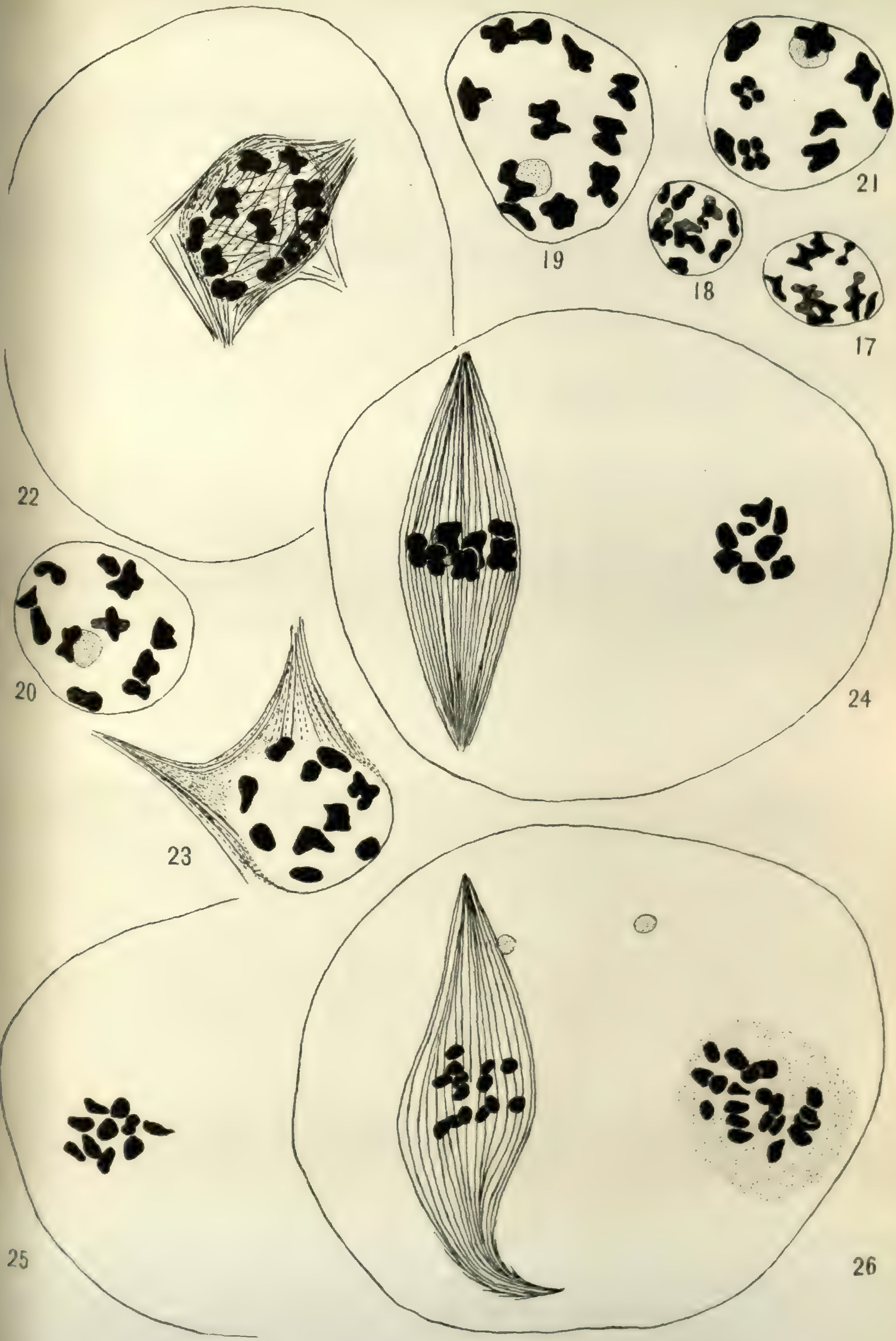

GATES on CHROMOSOMES of OENOTHERA

PLATE XIII

Figures in plates XIII and XIV from plants having 21 chromosomes; not numbered in developmental order.

FIG. 11.—Heterotypic spindle in side view, showing 20 or 21 chromosomes, not forming an equatorial plate, but loosely aggregated in the median region of the spindle; cf. *fig. 15*.

FIG. 12.—Telophase of heteroptypic mitosis, showing both daughter nuclei; one, uncut, shows 10 chromosomes.

FIG. 13.—Telophase, showing 11 chromosomes and a number of very small nucleoli; an unusual condition, probably due to the failure of the nucleoli to fuse at an earlier stage.

FIG. 14.—Telophase, showing 12 bivalent chromosomes, lying closely against the wall, tipped at various angles to the plane of view, which gives them a variety of appearances; an exceptional case, in which one chromosome too many has passed to the end of the spindle; 9 at opposite end; 2 nucleoli.

FIG. 15.—Metaphase of homotypic mitosis in polar view, showing equatorial plates of chromosomes, 10 chromosomes on one spindle and 11 on the other; cf. *fig. 11*.

PLATE XIV

FIG. 16.—Telophase of homotypic mitosis, showing three daughter nuclei; one chromosome left behind in the cytoplasm, leaving 10 chromosomes each in two of the daughter nuclei; many chromosomes show characteristic dumb-bell shape.

FIGS. 17, 18.—Very early telophase of the heterotypic mitosis, just after the nuclear membrane has been formed around the daughter nuclei; one shows 10 bivalent chromosomes, the other 11.

FIGS. 19, 20.—Telophases, each showing 11 bivalent chromosomes.

FIG. 21.—Telophase with 9 chromosomes only.

FIG. 22.—Prophase of the homotypic mitosis, showing 11 bivalent chromosomes, which have the same appearance as in the previous telophase; nuclear membrane just broken down, its position still occupied by a weft of fibrillae, against which the chromosomes lie; cones of the multipolar spindle forming.

FIG. 23.—Same stage as *fig. 22;* a less common condition, in which spindle formation begins on one side of the nucleus; 10 chromosomes, only a few of which show bivalent character.

FIG. 24.—Metaphase of the homotypic mitosis, showing one spindle in side view and the other in polar view; chromosomes not all shown.

FIG. 25.—Equatorial plate of homotypic spindle, showing 10 chromosomes in a single plane, few showing their bivalent character.

FIG. 26.—Early anaphase of homotypic mitosis, after division of the chromosomes; one spindle in side view, showing only part of the chromosomes (probably 20 in all); the other spindle in polar view, showing 20 chromosomes (two more are on this spindle in the next section); 2 nucleoli in the cytoplasm near the spindles.

THE DEVELOPMENT OF THE EMBRYO SAC OF *SMILACINA STELLATA*

F. McALLISTER

(WITH PLATE XV)

The object of this paper is to describe the development of the embryo sac of *Smilacina stellata* (L.) Desf., with a view to its possible bearing on the current interpretations of the lily type of embryo sac.

In 1880 TREUB and MELLINK (**27**) reported that in *Lilium bulbiferum* and in *Tulipa Gesneriana* the embryo sac mother cell develops directly into the embryo sac without any previous divisions.

In 1884 GUIGNARD (**11**) and also STRASBURGER (**24**) called attention to the fact of a reduction of the number of the chromosomes during the development of the germ cells of angiosperms. STRASBURGER (**25**) further pointed out in 1888 that in the case of the embryo sac in certain orchids and in Allium, the reduction of the number of the chromosomes occurs in the nucleus of the embryo sac mother cell.

It was further established simultaneously by OVERTON (**16**) and GUIGNARD (**12**) that, in the lilies and other plants in which the embryo sac mother cell develops directly into the embryo sac without previous division, reduction takes place in the nucleus of the young embryo sac. The natural conclusion from these discoveries is that the young embryo sac in these cases is the morphological equivalent of a pollen or embryo sac mother cell. That the nuclei resulting from the first two divisions in the embryo sac of the lilies are morphological equivalents of the microspores is strongly suggested by these results.

STRASBURGER (**26**) in 1894, discussing the formation of the embryo sac of the lilies, concludes that the cell in which the reduction of the chromosome number takes place is to be regarded as a mother cell and not simply as a young embryo sac, since in the ovaries of Allium and Helleborus he had found that the reduction of the chromosomes takes place in the embryo sac mother cell before it has

undergone division. He concludes that in these cases the course of development is abbreviated, so that there is no formation of reduced cells which are to be immediately absorbed, as is the case in many other species. According to this view the heterotypic and homeotypic divisions are transferred to the early stages of development of the gametophyte.

The term "macrospore" or "megaspore" is frequently loosely used by the supporters of this view in reference to the cell which develops into the embryo sac, whether it be the young embryo sac itself, or one of the daughter cells, or one of four granddaughter cells which have been formed by the reduction divisions.

From the evident homology of the nuclei of the first two divisions of the embryo sac mother cell of the lilies with the microspores, as suggested by the discoveries of GUIGNARD, STRASBURGER, OVERTON, and also later investigators, the obvious interpretation of their nature is that they are megaspores. According to this view the reduction divisions may be regarded as the sole criteria of spore formation.

STRASBURGER (**26**) further says, in reference to the significance of the number of divisions which intervene between the embryo sac mother cell and the completed embryo sac, with its egg: "That the number of these intervening divisions is not of primary importance is proved by the fact that the number is not always the same: thus in Lilium and Tulipa there are but three; in Ornithogalum, Commelyna, and species of Agraphis, there are four; in yet other cases the number is greater than five. . . . ."

Smilacina stellata, the species which I have studied, is a member of the order Convallariaceae. The data as to the morphological relationships of the genus Smilacina are scanty and in part contradictory. The order Convallariaceae is retained by ENGLER and PRANTL under the group Asparagoideae, but the nature of the relationship of the group to the other orders of the Liliales is not very clear. The other members of the Convallariaceae whose embryo sac development has been studied are Convallaria, Paris, and Trillium.

Convallaria majalis has been investigated, as to the development of its embryo sac, by WIEGAND (**29**). He reports that the embryo sac mother cell divides to form two fully separated daughter cells, the outer of which is the larger. The nuclei of both of these cells

undergo a second division, but this time no cell walls are formed. The resulting four nuclei again divide, and the partition wall between the two sets of nuclei disintegrates enough to allow a nucleus from the lower set to pass through and unite with one from the upper set. This fusion nucleus is the endosperm nucleus. If this account is correct, we have at least a partial absorption of a cell wall in the formation of the embryo sac and the utilization of all four nuclei of the double division, as will be described below for *Smilacina stellata*.

WIEGAND'S account is contradicted, however, by SCHNIEWIND-THIES (**21**), who reports that in *Convallaria majalis* the mother cell divides to form a row of four cells, one of which develops into the embryo sac, while the other three disintegrate. As a possible explanation of the difference which exists between her account and WIEGAND'S, she remarks that greenhouse material rarely shows normal development. WIEGAND, however, does not mention the use of such material in his investigation. That both WIEGAND and SCHNIEWIND-THIES are correct is possible, but not very probable. It is very much to be desired that this species be reinvestigated to clear up this confusion.

ERNST (**9**) reported that in *Paris quadrifolia* the lower of two daughter cells develops into the embryo sac. The upper daughter nucleus divides once, but the resulting nuclei degenerate. In the same paper he reports that the lower of two daughter cells of *Trillium grandiflorum* develops into the embryo sac. The upper daughter cell is smaller from the first and rarely divides. For *T. recurvatum*, on the other hand, CHAMBERLAIN (**6**) has reported that the embryo sac develops from the lower of four megaspores.

Though scanty, the literature on the order Convallariaceae suffices to suggest that there exists in the group great variation in the conduct of the cells and the nuclei resulting from the reduction divisions, and that we may here find transition conditions between the so-called normal type of embryo sac formation and that found in the lily.

The materials for this study were collected in the vicinity of Beloit, Wis., in May 1906 and 1907, in November 1907, and in June 1908. Flemming's strong solution gave the best fixation for the early stages, but worked badly for mature embryo sacs. With these mature embryo sacs the following chromacetic fixative gave good results:

chromic acid 0.7^{g}, glacial acetic acid 0.5^{cc}, water 100^{cc}. A fixative composed of two parts absolute alcohol and one part glacial acetic acid gave very good results also. Flemming's weak solution was not tried.

Smilacina stellata is especially favorable for obtaining a complete series of stages of the early development of the embryo sac. The flowers, eight to fourteen in number, are borne in a raceme, which does not expand till the embryo sac has nearly reached the eight-celled stage. It is therefore possible to cut an entire raceme longitudinally and get a maximum number of ovules cut parallel to their long axes. The oldest flowers are at the bottom. Each flower contains five to seven ovules, and about seventy racemes were cut in paraffin.

In the material collected November 16, the nucellus was only partly developed in the lower flowers of the raceme. There were no mother cells to be distinguished in any of this material. Material collected May 7 showed, in a few shoots, mother cells in the synapsis stage in the lower flowers, while at the top of the shoot the nucellus was barely differentiated. Most of the racemes taken at this date were farther developed than those mentioned above, the younger flowers being at least in the synapsis stage.

The mother cell is located at a variable depth beneath the surface of the nucellus. Not uncommonly it is immediately beneath the epidermis (*fig.* 1). In other cases it is separated from the epidermis by one cell layer. Most commonly two cell layers intervene (*fig.* 2). Measurements of several camera drawings of the mother cell at the synapsis stage gave an average breadth of 22 μ, and an average length of 30 μ.

I shall not here take up in detail the question of the reduction of the chromosomes. In the first division of the mother cell, thick double chromosomes, characteristic of the metaphases of the heterotypic division, appear (*fig.* 3). The number of bivalent chromosomes, as shown in the anaphase stage of this division, is twelve. The sporophytic number, as shown by root tip cells, is twenty-four.

A definite cell plate, and in most cases a cell wall, is formed, separating the two daughter nuclei of the first division before the second division takes place (*figs.* 4, 5). The cell plate is formed by

the thickening of the connecting fibers in the equatorial region to form a septum which separates the cells. This splits in the central region first, and often a thin layer of orange staining material is to be seen between the split layers. The plasma membranes formed by the splitting of the cell plate are made very conspicuous in cells in which there is a slight plasmolysis. The cell wall seems in most cases to be fully formed before the second division is completed. Several preparations were found in which the wall seemed to be incomplete after the second division (*fig. 7*). These, however, may represent a stage in the removal of the cell walls, as described below.

In many cases the wall of the first division is transverse, but not infrequently it is oblique (*figs. 4, 12*). The walls formed in the second division are extremely variable as to their position. Frequently (*fig. 11*) they are transverse, forming a linear row of four cells. Very often the outer daughter cell divides longitudinally and the inner transversely (*fig. 8*). Less frequently, the outer divides transversely and the inner longitudinally (*fig. 6*). *Fig. 7* shows an arrangement which is occasionally met with, both daughter cells having divided longitudinally. Rather frequently one section shows two cells divided transversely, while the next succeeding section shows two cells divided longitudinally (*figs. 9, 10*). This arrangement could only result from the division of the mother cell at first by a longitudinal wall parallel to the plane of the section, and the division of one of the daughter cells by a transverse wall and of the other by a longitudinal wall. This case is easy to recognize when the plane of the section is such that both of the cell boundaries of the second division are vertical. It is much more difficult to recognize when one of the cell boundaries of the second division lies in or near the plane of the section. Such a mode of division is of course frequently found in pollen mother cells. *Fig. 12* shows, however, by far the most common arrangement of the cells, in which the first wall is oblique and the walls of the dividing daughter nuclei are approximately at right angles to it.

Most of these various arrangements of the four cells resulting from the division of the embryo sac mother cell have been described by other authors, but in the stages immediately following we are

confronted with a series of changes leading to a method of embryo sac formation quite different from any which has been hitherto described.

The cell walls which separate the four megaspores break down and disappear. Immediately following the formation of four fully separated daughter cells and nuclei (*figs. 8, 11*) we find a stage in which the same four nuclei are seen occupying a large cell with no traces of cell walls separating them (*fig. 14*). The evidence of the disappearance of the walls between the megaspores is not based on scattered examples, selected from a large number of specimens, but on a large number of continuous series of stages taken from a single inflorescence.

Fig. 11 shows four cells fully separated by cell walls, while in the next older flower on the same shoot are found four nuclei in a common cavity (*fig. 14*). In *fig. 13* the cell walls formed by the second or homeotypic division have entirely disappeared, while a slight but distinct cleft, extending across the cell, shows the location of the wall formed between the nuclei in the first division.

A statistical examination of a number of racemes to determine the exact stage of development of the mother cell or its products in each ovule, gave very conclusive evidence of the continuity of these series. The following is a fair example of an average shoot of the proper age. In this shoot of nine flowers the youngest flower had mother cells in a stage later than synapsis. The next older flower contained mother cells in the prophase and metaphase stages of the first division. The third showed in one ovule a heterotypic anaphase stage, and the other ovules showed the heterotypic telophase stages. The fourth flower showed the earlier stages of the second or homeotypic division. The fifth contained daughter nuclei of the second division in the telophase stage, and the sixth showed nuclei of this division separated by cell walls. The next older flower showed in a part of its ovules the four nuclei still separated by cell walls, while the rest of its ovules showed little or no trace of cell walls between the four nuclei. The ninth flower, the oldest on the raceme, showed four-celled embryo sacs in all its ovules, with no traces of cell wall separating the nuclei. The series here described shows no trace of the growth of one megaspore at the expense of the other three, or of the

first and second divisions of such a megaspore to form a four-celled embryo sac. We must conclude, therefore, that the walls between the four megaspores break down, and their nuclei become the first four nuclei of the embryo sac.

In the succeeding stages there is a continuous growth of the nuclei resulting from the reduction divisions and of the embryo sac formed from these nuclei. Very soon after the cell walls have disappeared from between the reduction nuclei, vacuoles begin to appear in the young embryo sac. *Figs. 15, 16, 17* illustrate the gradual enlargement of the cell containing the four nuclei, and the appearance of vacuoles in the cytoplasm. The vacuolization more than keeps pace with the enlargement of the embryo sac, and finally the separate vacuoles unite to form one large centrally located vacuole. It is at about this stage of growth that the third division takes place, forming the eight nuclei of the complete embryo sac (*fig. 17*). The embryo sac still continues to enlarge. The nuclei remain unchanged for a relatively long period. The polar nuclei come together and lie in contact during this period of quiescence, but are seen to be fused before the cells in the micropylar region show any signs of differentiation to form the egg apparatus. Not until nearly time for pollination is there any rearrangement of these cells, which are to form the egg apparatus.

When finally differentiated the synergids are pear-shaped and faintly striated. The egg is somewhat larger than the synergids, and usually has a large vacuole in the basal region. It stains less heavily than the synergids. I shall not here discuss the formation of the cell boundaries of the synergids and the egg. The antipodal nuclei are smaller than the others, and usually occupy a constricted region at the lower end of the embryo sac. These antipodal nuclei at this stage often number more than three, and in such cases are usually separated by division walls.

The most natural interpretation of the phenomena just described is that the first four cells formed by the division of the embryo sac mother cell are megaspores, and that these four spores jointly combine to form one embryo sac. It would seem that this assumption is the only one possible, for before the division membranes disappear, the four cells conform to all the criteria for spores in other similar

cases, and the mere loss of the division membranes cannot affect their morphological value.

Some preliminary studies of *Smilacina racemosa* seem to show that the two outer nuclei, formed in the double division of the embryo sac mother cell, undergo two further divisions to form the eight nuclei of the mature embryo sac. I shall describe this species, with other related species, more fully in a later paper.

Recent studies have brought to light an increasing number of so-called atypical methods of embryo sac formation, which must be considered in attempting its phylogenetic interpretation.

In Eichhornia, according to SMITH (**22**), a cell plate is rarely formed between the two nuclei resulting from the first division of the embryo sac mother cell, and a cell plate is also rarely formed between the daughter nuclei of the second division. Reduction takes place and one of the four megaspores forms the embryo sac.

CAMPBELL (**2, 3**) reported the discovery of the 16-nucleate embryo sac of *Peperomia pellucida*, and in the following year JOHNSON (**13**), working independently, published an account of the same species. The embryo sac mother cell develops directly into the embryo sac. The sixteen nuclei organize to form an embryo sac with an egg apparatus consisting of an egg and one synergid. Six nuclei are cut off singly around the periphery of the embryo sac, and the remaining eight nuclei fuse to form the endosperm nucleus. Later JOHNSON (**14**) reported that in *Peperomia hispidula* fourteen out of the sixteen nuclei of the embryo sac unite to form the endosperm nucleus.

In contrast with the large number of nuclei in the embryo sac of Peperomia, is the embryo sac of *Helosis guayanensis*, which is reported by CHODAT and BERNARD (**7**) to contain only four nuclei when mature. This 4-nucleate embryo sac is due to the disintegration of the lower nucleus of the first embryo sac division, so the products of the division of the upper nucleus alone enter into the embryo sac structure.

Avena fatua has been shown by CANNON (**5**) to form its megaspores similarly to Eichhornia. Commonly no cell walls are formed between the four spore nuclei, but the lower of these nuclei develops into the embryo sac and the other three degenerate.

A case resembling that of Avena and Eichhornia is reported as

occurring in Crucianella by LLOYD (15). He reports that in three species of Crucianella investigated, four megaspores were formed which were not separated by cell walls, and that "only occasional exceptions could be found to this." The *upper* of these four nuclei develops into the embryo sac, and the three lower finally degenerate. Not uncommonly these three lower nuclei undergo division. Many cases were seen, LLOYD reports, in which the four megaspores had each divided once, thus forming eight nuclei in a common cavity. He says: "If these divisions are regarded as the first mitoses of an embryo sac we have four embryo sacs lying tandem." Only the one lying adjacent to the micropyle attains full development. Although he reports the disintegration of the lower megaspores, his figures admit other interpretations. As late as the third division of the functional megaspore, what he interprets to be one of the three lower megaspore nuclei is shown dividing in the same cavity with the four dividing nuclei of the embryo sac. The dividing nuclei seem to have the same appearance in every way. This would certainly show a prolonged activity on the part of these three inner megaspores. This delayed germination of the lower megaspores and the similarity of the dividing nuclei in every way, open up many possibilities. The exact fate of the eight free nuclei formed by the division of all four of the megaspores is left unsettled. LLOYD concludes that the outer megaspore attains full development, and that the eight nuclei therefore never organize to form an embryo sac jointly. This is a most interesting case, and a complete history of the embryo sac from the mother cell may throw light on the interpretation of multinucleate embryo sacs.

This omission of the division walls between the megaspores of a tetrad is found by LLOYD also in Asperula, which is related to Crucianella. Any one of four spores may germinate to form the embryo sac, and the other three finally degenerate. The three spores which do not germinate are at times difficult to distinguish from antipodal nuclei.

An important conclusion to be derived from the behavior of Eichhornia, Avena, Crucianella, and Asperula is that the division walls are not essential to the individualization of a spore, and that the failure to form division walls between the megaspores does not

necessarily result in the lily type of embryo sac, even though the nuclei seem to be of the same size and vitality. So long as the spore retains its individuality as such, we can expect each spore to develop into a separate gametophyte, and not till this individuality is lost can we expect to find more than one spore entering into the structure of a single gametophyte. This absence of division walls, however, may very well lead directly to such joint organization of a gametophyte as we find in Smilacina.

LLOYD, in discussing the nature of the first four nuclei of the embryo sac of the lily, is in favor of calling them spores. "But after all," he says, "spores in the sense meant here are equivalent to vegetative cells of a somewhat special sort, with no necessarily separate existence teleologically speaking." He urges against the idea that in the lilies the gametophyte begins with the mother cell this: "It would seem more natural to regard the gametophyte as an individual by coalescence, having its origin in four like vegetative cells whose primitive function has been lost."

In *Pandanus Artocarpus* and *P. odoratissimus*, according to CAMPBELL (**4**), the mother cell develops directly into the 14-nucleate embryo sac. The two outer nuclei formed by the reduction divisions do not divide, while the two inner nuclei divide to form twelve nuclei. A differentiation of the antipodal cells was reported, but no certain evidence of nuclear fusions to form the endosperm nucleus was found.

In 1907 PORSCH (**20**) proposed the theory that the two cell groups in the opposite ends of the angiosperm embryo sac are both to be interpreted as archegonia. The synergids are thought to correspond to two neck canal cells, and the upper polar nucleus to the ventral canal cell of the archegonium. According to this theory the embryo sac of Helosis consists of only one archegonium, the other having been suppressed. The behavior of *Smilacina stellata* perhaps supports this theory, in so far as it shows the equivalent origin of the two groups of nuclei which PORSCH interprets as archegonia.

Miss PACE (**19**) has reported that Cypripedium forms a four-celled embryo sac by two divisions of the lower of two "megaspores." This embryo sac may be interpreted by PORSCH's theory to be a single archegonium.

WENT (**28**), in a recent article on the Podostemaceae, reports that

in Oenone and Mourera a four-celled embryo sac is formed, similar to that in Helosis. The mother cell following synapsis divides to form two daughter cells, the upper degenerating. The lower daughter cell (which he calls the "megaspore") divides, and the innermost nucleus shrinks immediately to a shapeless mass of chromatin that is visible for a long time. This inner nucleus, though one of the first four nuclei formed by the division of the embryo sac mother cell, is interpreted by WENT as a nucleus of the embryo sac and not a spore. The nucleus remaining from the second division divides twice to form the four-celled embryo sac. The lower nucleus of this embryo sac degenerates, leaving only the three nuclei which form the egg apparatus. WENT notes that his results support the theory of PORSCH, except that the ventral canal cell is on the wrong side of the egg.

ERNST (**10**) has expressed the opinion that the 16-nucleate embryo sac of Gunnera, as described by him, consists of two of the archegonia of PORSCH in the chalazal end of the embryo sac and one in the micropylar end. Four nuclei in the central region of the embryo sac fail to form an archegonium, and unite with a polar nucleus from each archegonium to form the endosperm nucleus.

STEPHENS (**23**), in a preliminary note on certain Penaeaceae, reports that the sixteen nuclei of the embryo sac become divided into four groups, which lie at some distance from one another against the wall of the embryo sac. Three nuclei out of each group of four organize what appears to be an egg apparatus, while one nucleus from each group acts as a polar nucleus, and the four unite to form the endosperm nucleus. On PORSCH'S theory these four groups would represent four archegonia.

These examples seem to indicate a tendency of the nuclei of the angiosperm embryo sac to form groups of four. The evidence in favor of the archegoniate character of these groups, however, seems to me to be insufficient as yet. The relationship between the angiosperms and the gymnosperms is so remote that a comparison of the embryo sacs of the two groups with a view to homologizing the groups of nuclei associated with the egg is very difficult.

COULTER (**8**) further defends the idea that the first four nuclei of the embryo sac of the lily are megaspores, or "at least their nuclei."

His evidence lies in the fact of their being the product of the two reduction divisions. Hence they must be "megaspore nuclei, to be recognized as such by their cytological history and structure." The 16-nucleate embryo sacs of *Peperomia pellucida* are considered as resulting from two divisions of each of these megaspore nuclei, and the 8- and 16-nucleate embryo sacs, which JOHNSON reports as occurring in *Peperomia hispidula*, are regarded as formed by one division of each of the four megaspore nuclei to form the 8-nucleate embryo sac, and by two divisions of each to form the 16-nucleate embryo sac.

COULTER is inclined to regard it as a fundamental law that the angiosperm embryo sac is formed from the mother cell by never more than five nuclear divisions, the reduction divisions and three divisions of a megaspore. He concludes that the large number of nuclei in the embryo sacs of Peperomia and Pandanus may originate by the participation of more than one spore in their organization. This implies, further, that any embryo sac formed from one spore which has sixteen or more nuclei can be considered as relatively primitive, since it would require more than five nuclear divisions to produce it from the mother cell.

I do not see why the cases of the proliferation of antipodal nuclei should not be given more weight in the evidence. There is certainly an abundance of cases among the grasses and in the Ranunculaceae, as well as in other families, in which the innermost nuclei of the embryo sac are the product of more than five divisions previous to fertilization. It seems doubtful whether the embryo sac with more than eight nuclei can be explained on any such simple hypothesis, and it is to be remembered that, while there is apparently a physiological necessity back of the double division, there is nothing, as STRASBURGER has noted, in the phylogeny of the angiosperms which would explain or give special significance to a fivefold division.

BROWN (I) reports that evanescent cell walls are formed separating the first four nuclei of the embryo sac of *Peperomia Sintensii* and *P. arifolia*, in both of which the mother cell develops directly into the embryo sac. Though evidently convinced that in Peperomia these first four nuclei are megaspores, BROWN seems to object to the adoption of this explanation for the lily type of embryo sac in general.

Referring to the number of divisions which archesporial cells undergo to form the mother cell, he says: "Since we can trace the reduction of these divisions until, among the angiosperms, the archesporial cell may, without dividing, form one megaspore mother cell, it does not seem reasonable to suppose that the divisions of the mother cell to form four megaspores may not also be left out, and the mother cell function directly as a megaspore." Any analogy, however, based upon the archesporial cell, has against it that this latter cell is a doubtful morphological unit.

It cannot be maintained that any morphological interpretation yet proposed satisfactorily explains all the diverse types of embryo sac formation which have been described. That there are so many variations in important particulars gives ground for the expectation that further study may fill up many gaps in our current interpretations.

It is plain that within the group of the Convallariaceae there are types which go far toward explaining the origin of the old and familiar lily type of embryo sac. It is certainly plain that in *Smilacina stellata* four megaspores are formed, which are unmistakably separated by cell walls and subsequently recombine to form the first four nuclei of the embryo sac.

All the evidence favors the view that the first four nuclei of the lily embryo sac are morphologically, as well as from the standpoint of the reduction divisions, to be interpreted as megaspore nuclei.

SUMMARY

1. The mother cell of *Smilacina stellata* divides twice to form four nuclei, which are separated by walls to form four megaspores.

2. The division walls and plasma membranes which separate the four nuclei are absorbed, so that the four reduction nuclei occupy a common cell cavity.

3. Each of these four nuclei divides again, and the resulting eight nuclei organize to form the embryo sac.

4. It is plain from these facts that we have four individual megaspore cells combining to form one embryo sac or gametophyte in *Smilacina stellata*.

5. It is thus strongly suggested that in the embryo sac of the lilies the first four nuclei are morphologically megaspores.

6. In *Smilacina racemosa* the outer daughter cell of the heterotypic division develops into the embryo sac, although the long persistence of the two nuclei formed by the division of the inner heterotypic nucleus might suggest that they should be considered a part of the embryo sac. *Smilacina racemosa* shows temporary cell division at the close of the homeotypic division, agreeing in this respect with *Smilacina stellata*.

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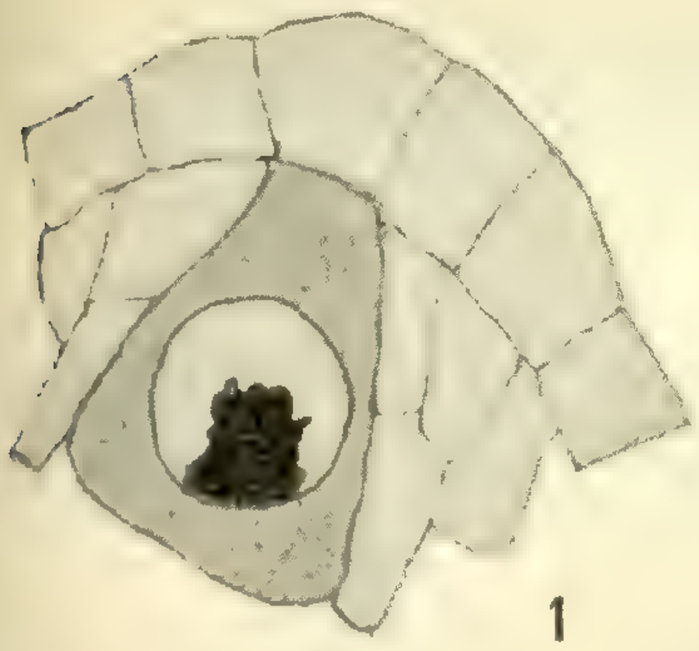
EXPLANATION OF PLATE XV

All figures were drawn with the aid of a camera lucida. The magnification in all the drawings is 670 diameters. The micropylar end is upward in each drawing.

FIG. 1.—Mother cell in synapsis stage; without tapetal cell.

FIG. 2.—Mother cell in prophase stage of first division; two cell layers between the epidermis and the mother cell.

FIG. 3.—Early metaphase stage of the mother cell, showing thick double chromosomes characteristic of first reduction division.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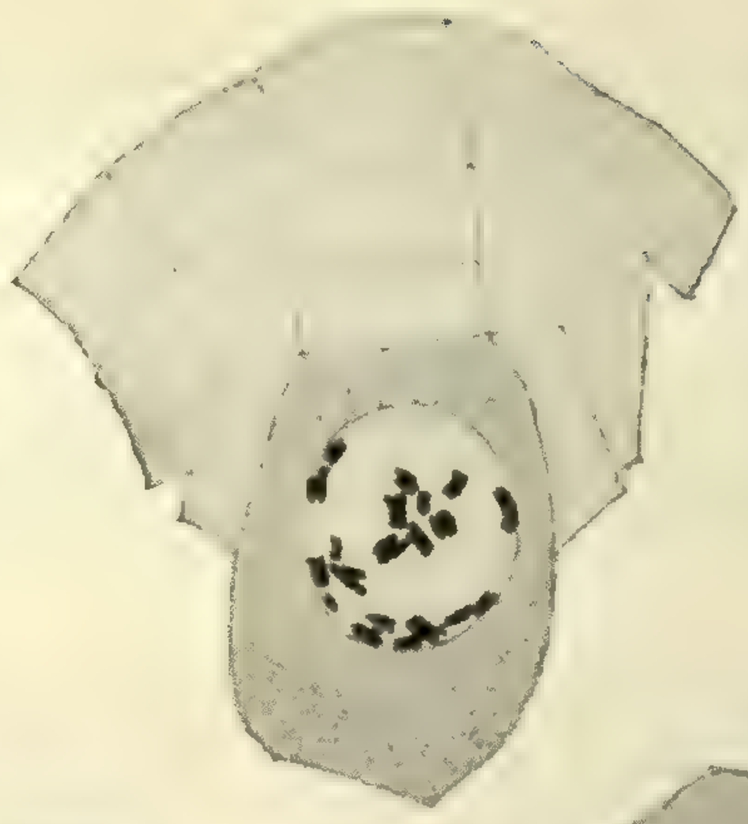
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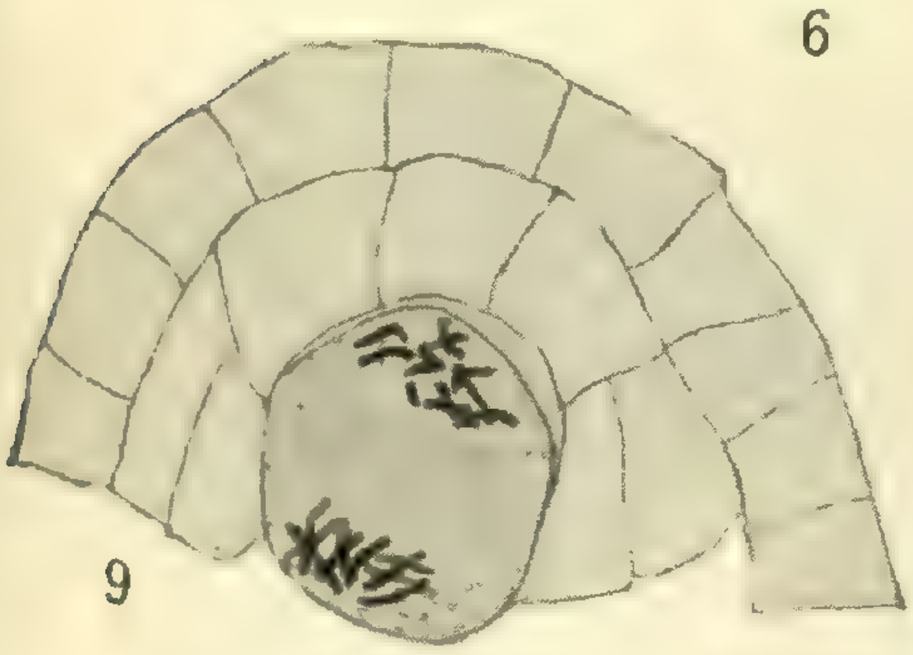
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McALLISTER on SMILACINA

FIG. 4.—Formation of cell plate and daughter nuclei at the close of first division of mother cell.

FIG. 5.—Early metaphase stage of homotypic division; axes of spindles oblique.

FIG. 6.—Daughter nuclei and cell plates forming at the close of second division; outer daughter cell dividing transversely and inner one longitudinally.

FIG. 7.—Nuclei of second division fully formed, arranged bilaterally; division membranes of second division do not reach outside walls.

FIG. 8.—The outer daughter cell has divided longitudinally and the inner one transversely.

FIGS. 9, 10.—Two successive sections of the same nucellus, showing the division of an approximately spherical mother cell; heterotypic division longitudinal; one daughter cell in the homeotypic division dividing longitudinally and the other transversely.

FIG. 11.—Row of four very large megaspores, with the division walls very indefinite.

FIG. 12.—Commonest arrangement of megaspores, in which first division is oblique and second at right angles to first; walls have disappeared but plasma membranes still persist.

FIG. 13.—Four megaspores; a cleft between two middle nuclei is all that remains of division membranes of cells.

FIG. 14.—Four megaspores, with no traces of cell walls, forming four-celled stage of embryo sac.

FIGS. 15, 16.—Stages in the vacuolation of the four-celled embryo sac.

FIG. 17.—Eight-celled embryo sac, showing micropylar and antipodal groups of nuclei, the two polar nuclei already separated out.

A STUDY OF PIÑON PINE

F. J. PHILLIPS

GENERAL DISTRIBUTION

No other tree species of the southern portion of the Rocky Mountain region presents more difficult problems in maintaining and reproducing the natural stands than does the piñon pine (*Pinus edulis*). It ranges from northern Mexico to eastern Utah, and Colorado Springs, Colorado. In an east-and-west direction it extends from the hills of western Texas to California. Along the northern and eastern borders of its range it is shrublike and of botanical importance only. In southern Colorado, Arizona, and New Mexico, it has a great economic and silvicultural importance, which will steadily decrease unless measures are taken to prevent excessive utilization.

It is commonly found in mixture with the one-seeded juniper (*Juniperus monosperma*) in the northern part of its range and with the alligator juniper (*Juniperus pachyphloea*) and one-seeded juniper in the south. Throughout its distribution it is associated with western yellow pine (*Pinus ponderosa*) and the scrub oaks (*Quercus Gambelii* and *Quercus acuminata*), often forming with these species a transition belt between stands of juniper and western yellow pine. Occasionally it is found with stunted Douglas fir (*Pseudotsuga taxifolia*). In association with the junipers, it forms the distinct woodland type so characteristic of New Mexico and Arizona, which in this region covers a more extensive area than any other forest type, and in which the piñon is decidedly the most important tree. It is occasionally seen in pure stands over small areas, but this is rare.

LOCAL OCCURRENCE

The tree thrives best at a general elevation of 1650 to 2350^{m} (5400 to 7700 feet) on moderate to steep mountain slopes and over broad, level, or sloping mesas. Small isolated specimens were found up to an elevation of 2600 and 2750^{m} (8500 and 9000 feet), while occa-

sional specimens may be found even higher than this. The best stands are found on coarse gravel, gravelly loam, or a coarse sand, of 1.5^{m} (5 feet) or more in depth, on which humus and ground cover are almost entirely lacking. The species often occurs on rocky areas, where the soil is only 15 to 30^{cm} (6–12^{in}) in depth, and frequently it is found growing in rock crevices. It is one of the first trees to gain a foothold on the lava overflows which are known throughout the southwest as *mal pais*. This rock in its disintegrated form supports fair tree growth, but even before disintegration has progressed very far, the junipers and piñon may be found encroaching upon it.

Another encroachment form of the piñon is to be found on small mounds which rise 0.6 to 3^{m} (2 to 10 feet) above the general level of the desert-like tableland at approximately 1500^{m} (5000 feet) elevation. On such islands as these, the piñon and one-seeded juniper take possession and maintain a limited growth. The same feature is noted at the bases of the hill and mountain slopes which bound these tablelands. This remarkably distinct tension line seems to be due to a greater soil porosity, less grass growth, and a smaller alkali content, which are manifest in slightly higher elevations. The distribution of these trees on such small mounds and limited in such a distinctive manner presents an ecological problem for future investigation.

On slopes where site conditions are favorable for western yellow pine, the piñon usually occupies the south and west aspects. Where conditions become less favorable, it occupies the north and east slopes, while the south and west slopes are bare or nearly so. This ability to stand poor conditions is also shown on a large number of mountain slopes ranging from 2830 to 3135^{m} (6000 to 7000 feet) in elevation, where scattering Douglas fir, of scrubby growth and badly affected with witch's broom, is found in the cañons; western yellow pine on the middle slopes; and piñon on the ridges and upper slopes, where the soil is scant and the soil moisture low.

A distinctive peculiarity was observed between Servilleta and Taos, New Mexico, in an open stand of the species in which approximately two-thirds of the trees have constricted bases at the surface of the ground. This constriction amounted to an average of 19^{mm} (0.75^{in})

in radius, but was occasionally noted where it amounted to 38^{mm} (1.5^{in}). Such a constriction is often seen on individual trees in nearly any stand, but in no other case was it found to be a stand as characteristic as it was near Servilleta.

Piñon is also resistant to severe climatic conditions, since it will succeed over severely exposed slopes where the average annual precipitation is less than 33^{cm} (13^{in}) and where evaporation and transpiration are high because of the semi-arid climate, the large amount of sunshine, and the prevalence of winds. In this respect it is undoubtedly the most resistant pine in the southwest. However, it prefers a slightly greater precipitation and areas less exposed to the wind. An example of the unfavorable influence of strong winds and a close-textured soil was noted in the vicinity of Fort Stanton, New Mexico, where a level plateau of nearly 8^{km} (5 miles) in length did not support a single tree, while similar plateaus on all sides, with less wind sweep and a coarser soil, showed luxuriant growth of both the piñon and the juniper. The tree does not live as long as the junipers, and in general is less resistant to unfavorable climatic conditions. In the drought which occurred in New Mexico from 1889 to 1904, piñon suffered considerably more than the junipers. Many mixed stands were observed in New Mexico and southern Colorado in which 75 to 95 per cent. of all dead trees were piñon. In the frost which occurred in April, 1907, piñon was affected, while the junipers resisted practically all injury. In the wet freezing snow of October, 1906, which caused immense damage to the forests of the southwest, fewer branches were broken from the piñon than from the brittle junipers.

The tree is also more resistant to disease than most of the conifers with which it associates. It is much less affected by the so-called false mistletoe (Razoumofskya) than is the western yellow pine and the junipers. It has fewer insect enemies than the western yellow pine, and is not affected by the witch's broom as is often the case with Douglas fir in the southwest.

TOLERANCE AND FORM

Piñon is distinctly an intolerant tree. During its seedling stage it prefers a moderate shade, and hence reproduces best under the

shade of older trees. After the seedling stage is passed it prefers the open, and is one of the most intolerant of forest trees. This gives an orchard-like appearance to most stands of this species. Occasionally stands of 0.7 density were noted, although few stands have more than 0.6 density.

On the best sites the trees reach a maximum height of 12 to 13.7^{m} (40 to 45 feet) and a diameter of 60 to 75^{cm} (2 to 2.5 feet) at breast height, but ordinarily the mature individuals range from 3 to 10.5^{m} (10 to 35 feet) in height and from 15 to 45^{cm} (0.5 to 1.5 feet) in diameter. A difference in development was apparent on different sites. On exposed sites the tree is globular, very scraggly when mature, and has little or no clear length. On favorable sites trees in the open have a very short clear length and a fairly regular globular or egg-shaped crown. If grown in stands, the trees have a greater clear length and a flat or vase-shaped crown. Young trees on favorable sites are conical or globular in shape and usually very regular in form.

On the most exposed sites, shrublike trees were found which were fifty to eighty years old, and only 1.8 to 3^{m} (6 to 10 feet) in height, with a crown diameter reaching a maximum of two to four times the height of the tree. On such trees it was impossible to distinguish the leader from the branches, and the general appearance of the tree was much like that of the dwarf mountain pine (*Pinus monticola*). The foliage is more densely clustered on these dwarf trees than it is on trees in the open, with shorter and apparently thicker leaves. Practically all trees, whether growing on poor or good sites, are characterized by dead and half-dead branches, which are retained on the tree for several years. This is characteristic of nearly all species in the southwest and is due to the small amount of growth that is made, the necessity of retaining only a small amount of living tissue, and the dry nature of the climate, which allows the retention of dead branches for a longer period than would a moist climate. In exceptional stands, such as occur to the west of Servilleta, New Mexico, where a clear length of 4.5 to 7.6^{m} (15 to 25 feet) is not exceptional, the branches are shed largely because the density of stand prevents the formation of as large branches as are found in those trees which enjoy full sunlight.

WOOD

Piñon wood is moderately heavy for the pines. It is used extensively for fuel and has been limitedly used for fence posts, telephone poles, corral posts, mine lagging, railroad ties, charcoal, and inferior lumber. Some authorities have recommended its use for fence posts, but this is to be seriously questioned as it has little durability in contact with the soil, and even the natives are discarding it for such use. It may be rendered valuable, however, by the use of preservatives. The tree is remarkable in its fuel value, and its use for such a purpose should be greatly encouraged. It is a common practice to cut branches or trees after they have been dead about two years. If cut before this time, the wood has not seasoned sufficiently to burn readily. If cut after this time, it has usually deteriorated to some extent. As a hearth fuel, it is not surpassed by another conifer and by only few hardwoods. It starts to burn readily, retains fire for a considerable length of time, gives a large amount of heat, and does not throw sparks. Since open fires are very common in this region, this wood serves an excellent purpose. Sample acres which have been clear cut have given a yield of 180 to $360^{\text{cu m}}$ per hectare (20 to 40 cords per acre), while extensive stands have averaged 90 to $108^{\text{cu m}}$ (10 to 12 cords).

FRUIT

The young cones are dark red and occur in elongated clusters. The pistillate form is easily distinguished by short stalks. Both sorts are very plentiful in seed years, but are scarce during other years. The mature cone is short, top-shaped, 19 to 50^{mm} (0.75 to 2^{in}) long and often as broad as long. The cones open on the tree and are covered by a large amount of free resin, which makes them difficult to handle. They often occur on trees only 0.9 to 1.2^{mm} (3 to 4 feet) in height, which are ten to twenty years old, but the best crops are borne on mature trees which produce 35 to 280^{l} (1 to 8 bushels) of cones; each cone contains two to thirty seeds, with an average of ten to twenty seeds. The trees have been known to yield 336^{kg} of seed per hectare (300 pounds per acre), while a much larger area has been known to produce an average of 73^{kg} per hectare (65 pounds per acre).

Seed years usually occur at five-year intervals, but have been reported at shorter intervals than this. The seed is well rounded at the base, tapering with prominent ridges to an acute point. It is usually dark brown on the lower side, with more or less mottled orange yellow on the upper side, 9 to 12.5^{mm} (0.375 to 0.5^{in}) long, 6.5 to 9^{mm} (0.25 to 0.375^{in}) broad, with a thin shell which cracks most easily along the line of the most prominent ridge. The seed wings are about one-half the length of the seed, easily detached, and of no practical use in seed distribution. The seeds usually have a high percentage of infertility, which varies from 5 to 20 per cent., but in one case went as high as 85 per cent. Poor seeds are often lighter in color than good seeds. Germination power is lost very readily, which necessitates special storing when they are to be used for artificial planting, and good site-conditions when the stands are to be reproduced naturally. It is a matter of note that the seeds from the northern portion of the range are usually considered better than those from the south. Five samples collected in various localities gave the following results:

No. per pound (453.6 gm)	Percentage viable; knife test	Percentage viable; water test	Percentage viable; in greenhouse	Percentage viable; in open	Where collected
2510	87.2	84.0	82.2	75.6	Ft. Bayard, N. M.
2215	89.1	86.6	80.3	69.2	Tres Piedras, N. M.
1810	91.2	86.0	78.1	70.4	Ft. Garland, Col.
1950	92.7	88.5	81.3	71.0	Ft. Garland, Col.
1520	99.2	97.1	96.4	90.3	Lincoln, N. M.

Weevils sometimes affect the seed before the cones open. Birds and rodents eat the seed extensively, and stores are made by mountain rats which were found to contain a maximum of 35 to 70[l] (1 to 2 bushels) of clean seed. Ants are known to eat seed, especially at lower levels. In the early days, the Indians and Mexicans used the piñon as a staple article of food. At present, it is gathered in immense quantities and sold as a delicacy. It is eaten most extensively in and about the region where the tree grows naturally, but large amounts are being sold at fruit stands throughout most of the United States. To prevent the seeds from spoiling and to retain flavor, they are usually baked immediately after being gathered.

Most of the seeds are collected by Mexican women and children, who usually spread a sheet or blanket on the ground and then shake or pound the tree and its branches until the seeds fall from the open cone. Later in the season, the seeds are picked up by hand from the ground beneath the trees. In the best part of the seed harvest, enough are gathered by single families to be sold by the grain bag full or the wagon load. Since the Mexicans take almost no precautions against the spreading of smallpox, it is said that the worst ravages of the disease occur during a seed year of the piñon. Single dealers have been reported as having bought 9000 to 21,500^{kg} (20,000 to 50,000 pounds). The delicate flavor of the seed makes it a favorite, and an extensive market is being rapidly developed for it. During seed years the native collectors sell it at the rate of five to fifteen cents per pound, according to the ease of collecting the seed and the proximity of the market, while dealers in many of our cities sell the seed at a rate of forty to sixty cents per pound.

REPRODUCTION

Natural reproduction is limited because of the infrequency of seed years, unfavorable climatic conditions, infertility of seed, rapidity with which the seed loses its germination power, loss of seed eaten by rodents, birds, and man, and unfavorable site-conditions. Grazing interests are also a factor in limiting the reproduction of the species, since sheep, cattle, and goats are grazed throughout its entire distribution. It is apparent to even the casual observer that extremely large areas are not reproducing themselves, yet owing to the difficulties of site and the methods by which the tree may be reproduced, the problem of reproduction is an extremely difficult one, and one for which, at the present time, no adequate solution can be offered.

FUTURE MANAGEMENT

From the nature of the stand in the southwest, it is apparent that clear cutting would not be an advisable system, because of the exposure of the site and the difficulties of restoring the stand. On the other hand, the large amount of seed consumed by man and other agencies makes natural seeding exceedingly difficult, and even though grazing and fire are entirely eliminated, it is doubtful if satisfactory reproduc-

tion will be secured in even a bare majority of sites. Until the problem of reproduction is more thoroughly worked out, the policy should be to remove only the dead and dying piñon trees for fuel, thus allowing a careful management without encroaching seriously upon the natural stands as is being done at the present time. It would seem from the nature of the site that the stand could be made to succeed best by the selection system, consisting of the removal of the dying trees. The sale of this fuel with that of a large portion of the seed should furnish a moderate income. This production would be low, as contrasted with high-type coniferous forests in other regions, but when consideration is given to the value of this species for fuel and seed, the question of immediate returns is a minor one.

UNIVERSITY OF NEBRASKA

BRIEFER ARTICLES

ON THE DEMONSTRATION OF THE FORMATION OF STARCH IN LEAVES

For a qualitative demonstration of photosynthesis in starch-forming leaves it is advantageous to know the time in darkness required for the disappearance of accumulated starch, and the time in light required for its subsequent demonstrable formation. No general rule can be given for either, since the time required varies widely for the different species. Therefore, in continuation of the series of studies carried on in the laboratory of plant physiology of Smith College on the physiological constants of the educationally useful plants,[1] I have tried to determine these data for such plants, and also, by comparison, the best plants for the purpose. Throughout this paper I have used the expression "disappearance of starch" rather than "translocation." In a general way the processes, of course, correspond, yet the term "translocation" implies the removal of the starch from the leaf, while here we are dealing only with its disappearance as starch.

The method employed in the present study was as follows: Five actively growing plants of each species, always after a bright day and between 4 and 5 o'clock, were put in a dark room having a steady temperature of 18°–22° C. as recorded by a thermograph. Twice a day, about 9 A. M. and 2 P. M., leaves were tested for starch by SACHS's iodine method, and the time when all starch had disappeared was noted. No attempt was made to find the exact hour when the leaves were empty. This would necessitate testing them every hour during the night as well as during the day, and for the purpose of the present study, the results would be of little value. In the following table, therefore, the time in darkness required to empty the leaves of starch is given in night and day periods rather than in hours. For the iodin test the leaves were first boiled 1 minute to swell the starch, were blanched in warm alcohol, were put in water a few minutes to remove the alcohol and soften the tissues, and were then immersed in a solution of iodin. The solution used was 5^{gm} potassium iodid, 1^{gm} iodin, 10^{cc} water, to which, when dissolved, water was added to make 1 liter of solution.

Thus was the time of disappearance of starch determined with sufficient accuracy for all practical purposes. To determine the time required

[1] BOT. GAZETTE **40**:302. 1905; **45**:50. 1908; **45**:254. 1908; **46**:50. 1908. **46**:221. 1908.

for starch formation, it was found best to use some type of screen such that a sharp contrast would show between the light and dark parts; and for this purpose light-screen boxes were used. These boxes have been fully described by Professor GANONG in the BOTANICAL GAZETTE.[2] Briefly, they are small boxes made of white paper blackened inside, with a network of threads across the top to support the leaf, and holes near the bottom to allow the air to pass through freely. A glass plate covered with tinfoil having a pattern cut in it is held closely against the network by a wire spring. When in use the leaf is held between the glass plate and the network. The principal advantage of these screens is that while excluding all light, they allow nearly the normal access of carbon dioxid to the leaf. The caution, by the way, against using screens which cut off all carbon dioxid as well as the light has been made several times in recent years, but some of the new elementary textbooks are still copying the old and erroneous method of putting cork or tinfoil on both sides of the leaf. Several light-screen boxes were attached to the leaves which had previously been emptied of starch, and the plants were placed in strong diffuse light. Leaves were then taken off and tested for starch at 10-minute intervals. In order to compensate the effects of individual peculiarities, 5 plants of each species were tested. The results given in the table are for full-grown (except in the three cases noted), but not mature, leaves on actively growing plants, in pots, in a greenhouse. In the first column is given the time in darkness required to empty the leaves of starch; in the second, the time in diffuse light required to make enough starch to show a pale but clearly defined figure with the iodin test; in the third, the time required to show a sharply defined, dark figure; while in the fourth is given the time required for the iodin to produce its full effect.

The best leaves, obviously, for this study are those in which photosynthesis is most active, from which starch disappears most rapidly in darkness, from which the chlorophyll can be extracted quickly leaving the leaf white, and which give the iodin reaction quickly. As shown by the accompanying table, these are *Pelargonium hortorum zonale*, *Fuchsia speciosa*, *Senecio mikanioides*, *Impatiens Sultani*, and young plants of *Helianthus annuus*, *Ricinus communis*, *Phaseolus vulgaris*, *Zea Mais*, and *Cucurbita Pepo*.

On the other hand, some leaves are not good for this study. *Begonia palmata*, *Oxalis Bowiei*, and *Pelargonium peltatum*, when boiled to swell the starch, partially disintegrate, so that the figure does not show clearly with the iodin test. It is possible, of course, to apply the iodin test without previous boiling of the leaf, but it takes 24 to 48 hours according to the

[2] BOT. GAZETTE 43:277. 1907.

age of the leaf; besides, these leaves turn brownish yellow on the application of iodin and therefore do not show clearly the reaction with starch. *Ficus elastica* requires more than one day to make a perceptible amount. Only very young leaves of *Primula obconica*, *Primula sinensis*, and *Cineraria cruenta* can be emptied of starch; being stemless plants, they probably use the older leaves for its storage. Young plants of *Pelargonium domesticum* empty their leaves of starch in about 40 hours, but older plants are unsatisfactory, because, even after 4 days in darkness, there are still spots of starch in the mesophyll. All these plants, except *Oxalis Bowiei*, and the young plants of *Pelargonium domesticum*, require 3 to 5 days in darkness —too long a time for the subsequent good of the plant.

Name of plant	Disappearance of starch in darkness (T. 18°–22° C.)		Formation of starch in light (T. 20°–25°C.)		Iodin test
			Perceptible fig.	Good fig.	
	nights and days		minutes	minutes	minutes
Abutilon	2	1	30	120	5–10
Begonia coccinea	3	3	60	240	10
Brassica oleracea	1	0	20	50	5
Cineraria cruenta (young ls)	5	4	45	180	30
Coleus Blumei	2	1	30	50	3–8
Cucurbita Pepo	1	0	15	50	4–15
Euphorbia pulcherrima	2	1	60	240	20
Fuchsia speciosa	1	0	45	90	15–25
Helianthus annuus	1	0	30	120	5
Heliotropium peruvianum	1	0	45	120	5–10
Impatiens Sultani	1	0	30	120	5
Lupinus albus	1	0	60	240	3
Oxalis Bowiei	1	0	45	240	10–30
Pelargonium domesticum	2	1	50	240	40
Pelargonium peltatum	3	3	50	270	60
Pelargonium hortorum × zonale	2	1	20	50	10–15
Phaseolus vulgaris	1	0	20	90	5
Primula obconica (young ls)	5	4	120	240	5–10
Primula sinensis (young ls)	4	3	45	120	10
Raphanus sativus	1	0	35	60	3
Ricinus communis	1	0	20	60	5–15
Salvia involucrata	2	1	90	120	4
Salvia splendens	3	2	30	60	1–5
Senecio mikanioides	1	0	20	50	5
Senecio Petasitis	3	2	30	180	25
Tropaeolum majus	2	1	50	90	1
Vicia Faba	1	0	60	240	10
Zea Mais	3	2	30	120	5

Prolonged darkness produces two distinct deleterious results. First, some plants, as Heliotropium and Impatiens, after 3 or 4 days in darkness, drop their leaves, owing to some derangement of their mechanism, the

cause of which I have not investigated. Also, Tropaeolum leaves soon turn yellow in darkness. The second injurious result, and the most important in this study, occurs in nearly all plants. Some of the photosynthate is of course being constantly used in growth or for storage in the stem, and since the plant can make no more in darkness, the percentage of sugar in the cell-sap decreases more and more by diffusion into the stem. Then, as PFEFFER has shown,[3] when the plant is brought into the light no starch is deposited until this percentage of sugar is repaired. The following example is typical: Two plants of *Fuchsia speciosa*, apparently alike, were put in the light at the same time, after having been in darkness, the one 64 hours, the other 16 hours. Halves of leaves tested showed no starch. After 2 hours in the light, the other halves on the first plant showed much less starch than those on the second plant. To obtain the most rapid formation of starch, therefore, it is important that the plant should be kept in darkness only long enough just to cause the disappearance of starch.

The disappearance of starch is not always even. In Coleus, *Primula verticillata*, *Primula obconica*, and *Fuchsia speciosa*, the base of the leaf is emptied of starch before the tip. This agrees with what SACHS found in some other leaves.[4] So far as I have tested them, I have found this to be true only of ovate or oblong leaves. This may be correlated with the greater abundance of stomata near the base of the leaf. In round leaves like *Pelargonium zonale* and Tropaeolum, the starch seems to disappear evenly from all parts. The starch disappears from the young leaves on a plant before it does from those which are mature.

The effects of temperature on the amount of starch present are especially important. In several of my experiments, leaves of *Fuchsia speciosa*, *Euphorbia pulcherrima*, and *Pelargonium zonale* which were kept in direct sunlight 4 hours showed very little starch, while leaves on plants in diffuse light, at the end of 4 hours were full of starch. *Fuchsia speciosa* after being in diffuse light at 28°–31° C. for 3 hours showed only a trace of starch, while other leaves in 3 hours at 18°–20° C. appeared black with starch, with the iodin test. A comparison of these two sets of experiments shows that the small amount of starch present in leaves in direct sunlight is undoubtedly connected with their high temperature. In order to get the best results, therefore, from experiments in the formation of starch in the leaves of potted greenhouse plants, it is necessary to keep the plants at a temperature not exceeding 22° C., and to insure this it is well to keep them in diffuse rather than in direct sunlight. It will be of interest to compare these results with those obtained by SACHS for outdoor plants, as described in his classical

[3] Physiology 1:321.

[4] Ges. Abhandl. 360.

paper.[5] He found that the rate of the disappearance of starch from the leaves increases with the temperature. When the nights were very warm, some plants (Nicotiana, Phaseolus, Juglans, and others) completely emptied their leaves of starch in one night. But after cool nights, 6°–9° C., there was no perceptible loss of starch in some, and the disappearance was incomplete in others. SACHS found also that the amount of starch present at any time of the day is affected by temperature. At a temperature of 20°–25° C. the quantity of starch in the leaves increased steadily from morning until evening. But on hot afternoons at a temperature of 30°–35° C. the leaves of Helianthus contained less starch than in the morning at 8 o'clock. The reason for this phenomenon is found in the fact that translocation from the leaf into the stem increases with rising temperature more rapidly than photosynthesis. All of these considerations emphasize the important precaution that to obtain the best starch formation in leaves, the temperature should not be permitted to rise above 20°–22° C., which is apparently the optimum for this process, as it is for the best general health of such plants as are used in these studies.

Also it must be remembered that plants cannot give good results in this or any physiological experiment, when suffering from previous starvation, as in the case of pot-bound plants or those which have been kept in darkness for a long time; when suffering from over-stimulation from high feeding, or from being kept at too high temperatures; and when they have passed their grand period of growth. As a rule plants, and particularly annuals, are in their best condition just before flowering.—SOPHIA ECKERSON, *Smith College.*

THE MORPHOLOGY OF *RUPPIA MARITIMA*—A CRITICISM[6]

In section D, "Function of the root," GRAVES (p. 113) has wandered beyond the natural limits of his paper as a morphological study, and while the propriety of this is perhaps questionable, the basis for this criticism is that this section D contains a statement which I think is an unwarranted misinterpretation of some of my own writings, and also a statement that would leave most readers misinformed. I wish first to consider the following: "On the other hand, POND'S experiments[7] fail to show conclusively whether or not water and dissolved salts are absorbed by the part of the plant above

[5] Arbeit. Bot. Inst. Würzburg **3**:1 ff., as cited in Ges. Abhandl. 354–387.

[6] GRAVES, A. B., The morphology of *Ruppia maritima.* Trans. Conn. Acad. Arts & Sciences **14**:59–170. 1908.

[7] POND, RAYMOND H., The biological relation of aquatic plants to the substratum. U. S. Fish Commission Report **1903**:483–526. 1905.

the soil." Being somewhat uncertain as to the intended meaning of this statement, I have learned by correspondence that GRAVES believes that in my paper I have committed myself to the notion (in the absence of conclusive evidence) that the larger submerged and rooting water plants derive their mineral food exclusively from the soil, and that there is no absorption of mineral food by organs other than the roots. Such a notion I have never held and do not consider my paper as warranting or encouraging such an interpretation. My efforts to force certain species to live and grow in nutrient solutions and without a substratum were not continued to a satisfactory conclusion, and for that reason I remained noncommittal on the possibility of absorption by the other organs of the plant.

My general conclusion was that certain species which were tried were found to be dependent upon their rooting in the soil for optimum growth and cannot survive a single season if denied a substratum of soil.

Whatever the absorption by the organs other than the roots amounts to (in the species tried), it is so small that the species would probably become extinct if forced to depend upon it exclusively under otherwise natural conditions.

Looking at section D as a whole we find that every paragraph is general in its scope and treatment. GRAVES enumerates four reasons for not placing too much emphasis "on the absorbing capacity of the root." One of the reasons is "the total lack of branches and slenderness of the roots." I think any reader would infer that submerged plants have slender roots without branches. So far as I know the statement is correct so far as the "slenderness" is concerned but in my paper (*l. c.*) there is a section on factors influencing the development of lateral roots

Speculation as to the relative importance of absorption by the roots of submerged plants as compared with that in the case of land plants is of little value. I refer particularly to the statement by GRAVES as follows: "In brief, the absorption carried on by the roots of submerged plants and the importance of this function in the economy of the plant is much greater than is implied by SCHENCK—but, on account of the peculiar environmental conditions of submerged plants, it can never equal in importance the absorption of the roots of land plants."

If the submerged species are as dependent upon rooting in the soil as my results show, the so-called absorptive function of the root is a vital necessity and it certainly is not more than this for land plants.—RAYMOND H. POND.

CURRENT LITERATURE

BOOK REVIEWS

Bacteriology, general and special

Although bacteriology in the past decade or more has developed to such an extent as to warrant being considered more broadly than merely as an *Anhang* to medicine, yet the texts prepared for the use of biological students have been written, in the main, from a comparatively restricted point of view, usually being designed for medical students. Naturally, the limitations of time demand that the student in technical courses, such as medicine, shall receive specific instruction in the subject-matter relating to the activities of those organisms that are of especial moment to him; but in our larger universities the time is ripe for the presentation of the subject of bacteriology from the standpoint of the student of general science. A course of training in general bacteriology is as essential in supplementing a general course in biology as is general chemistry. The works of DE BARY and FISCHER served as a suitable foundation in their day for the treatment of bacteria from the biologic viewpoint, and there is great need that such works be brought down to date.

Dr. JORDAN, the author of a recent textbook of general bacteriology, has had an unusual opportunity, by training and position, to develop a strong course in this subject; yet one cannot help feeling that even in a university atmosphere, the needs of the medical student were uppermost in his mind in the preparation of his book. And this impression prevails in spite of the fact that the volume purports to cover the general field, and attempts to present the "perspective of the subject, with emphasis on general rather than on special questions." A casual inspection of the book gives the impression that it is in the main medical, but a more careful analysis shows that much more satisfactory treatment of the non-medical phases is given than is generally the case. About 70 of the 540 pages of text are devoted to the morphology and physiology of the bacteria. The addition of chapters on organisms of the soil, air, and water, as well as of milk and its products, and on bacterial plant diseases, widens the scope of the work, but it is very evident that the treatment in these special chapters is no so well balanced as is the presentation of the animal pathogens. Indeed, the work, as a whole, is so largely medical in its aspects that we feel that there is yet to be written a text on general bacteriology that will fairly present these organisms from the biological point of view. For example, it would seem that a subject of such biological and historical importance as fermentation should

[1] JORDAN, EDWIN O., A textbook of general bacteriology. 8vo. pp. 557. *figs. 163*. Philadelphia: W. B. Saunders Co. 1908. $3.00.

receive adequate treatment. Bacteriology will never attain its true position as a member of the sisterhood of biological sciences until some one of its devotees will sacrifice his time to prepare a comprehensive text that presents the bacteria in their relations as living things, rather than as capable of affecting prejudicially or otherwise man and beast. Special fields may well receive special treatment, with such summary of general matter as may be absolutely necessary to enable the special class of students to handle the subject.

While the volume fails somewhat to meet our anticipations and is hardly adequate from the biologic point of view, the admirable presentation of the medical part of the volume shows careful work. The addition of chapters on the pathogenic Trichomycetes, Blastomycetes, and Hyphomycetes, as well as the disease-producing protozoa and diseases of unknown etiology, will be helpful to the medical student in correlating many of the recent advances in microbiology. With the rapid widening of the scope of study relating to the microparasites, we shall soon be driven to the adoption of the French term "microbiology," rather than the more restricted German title of bacteriology.—H. L. RUSSELL.

The eight years which have elasped since the publication of the first edition of CONN'S *Agricultural bacteriology* have been years of rapid advancement in all lines of bacteriological activity. A second edition[2] finds much new material to be incorporated.

The author in his preface admits that the limitations of the term agr cultural bacteriology are puzzling. The farmer has most of the city problems of sanitation in miniature and many more in economic bacteriology. The prevention of infectious disease in man and animals; farm engineering with its problems of sanitation, water supply, and sewage disposal; the function of bacteria in the production of butter and cheese; the conservation of the soil by encouraging the growth of beneficial bacteria; the part which bacteria play in the curing and preparation of food for man and animal—these problems range over practically the whole domain of bacteriology. A text which attempts to cover such a field in a little more than three hundred pages must of necessity be an outline merely and not an exhaustive treatment of the subject. Each chapter could be easily elaborated into a volume.

In make-up the volume has improved. The pages have been reduced from 412 to 331 in number. The illustrations have been numbered and many old ones replaced by better. Part, at least, of the decrease in size is due to the elimination of the references to literature found at the end of each chapter in the first edition. The text has been entirely rewritten, though the main divisions have remained as before. The revision has resulted in increased conciseness and clearness of expression. The discussion of bacteria and the nitrogen problem is excellent for use in an agricultural high school, but seems rather inadequate as a presentation of the subject to college students with two or more years of training in chemistry

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[2] CONN, H. W., Agricultural bacteriology. Second edition. pp. x+331. *figs.* 64. Philadelphia: P. Blakiston's Son and Co. 1909. $2.00.

and biology. A chapter on bacteria and soil minerals has been added. Tuberculosis is the only disease which is treated at length. The treatment of acquired immunity is misleading, in that vaccination is the only method of conferring immunity which is discussed, and the natural inference is that diphtheria and other diseases are thus treated. Antitoxins are not mentioned. A part of a chapter on fungus diseases of plants shows plainly the effect of too much condensation. The characterization of wilts, rusts, etc., on page 295 is unscientific and inaccurate. The student can gain little by a mere list of names of hosts and parasites such as this chapter contains. There is much that might be eliminated to make room for more adequate treatment of other subjects. As an introduction to the subject for the general reader or for the high-school student the volume is excellent; as a college text, however, it seems inadequate.—R. E. BUCHANAN.

NOTES FOR STUDENTS

Current taxonomic literature.—R. HÖROLD (Bot. Jahrb. **42**:251–334. 1909) presents a synoptical revision of the American Thibaudieae and carefully tabulates their geographical distribution. One monotypic genus (*Englerodoxa*) and 67 species referred to 17 genera are published as new to science.—G. MASSEE (Annals of Botany **23**:336. 1909) has published a new genus (*Gibsonia*) of the Ascomycetes; the new fungus was found growing in a drain in North Lancashire, England.—E. A. FINET (Bull. Soc. Bot. Fr. IV. **9**:97–104. *pls. 1, 2.* 1909) describes several new or noteworthy species of Orchidaceae, some of which are American.—L. A. DODE (*ibid.* 232–234) has published a new genus (*Orias*) of the Lythraceae from China.—N. L. GARDNER (Univ. Cal. Pub. Bot. **3**:371–375. *pl. 14.* 1909), under the title "New Chlorophyceae from California," has published two new monotypic genera (*Endophyton* and *Pseudodictyon*); a new species of Ulvella is also proposed.—O. TSCHOURINA (Bull. Soc. Bot. Genève II. **1**:98–101. 1909) has published a new genus (*Astrocladium*) of the Palmellaceae. The new alga was discovered in the vicinity of Geneva, Switzerland, and is represented by a single known species.—W. BIALOSUKNIA (*ibid.* 101–104) proposes a new genus (*Diplosphaera*) of the Pleurococcaceae, to which is referred but one species. The alga was isolated from the lichen, *Lecanora tartarea*, and developed as a pure culture.—G. O. MALME (*ibid.* 182) records a new species of Xyris from Brazil.—V. CALESTANI (Nuovo Giorn. Bot. Ital. N. S. **15**:355–390. 1908), under the title "Sulla classificazione delle crocifere italiane," recognizes 31 genera for Italy, including one genus (*Euxena*) published as new to science.—R. PAMPANINI (Bull. Soc. Bot. Ital. **1908**:132–134) describes a new species and variety of Tithonia indigenous to Mexico.—K. K. MACKENZIE (Muhlenbergia **5**:53–58. 1909) lists several species of Carex collected by A. A. HELLER in Nevada in 1908 and describes two new species.—P. B. KENNEDY (*ibid.* 58–61. *pl. 2*) in continuation of his "Studies in Trifolium" describes and illustrates a new species from Oregon.—W. FAWCETT and A. B. RENDLE (Journ. Botany **47**:122–129. 1909) in continuation of their studies on Jamaica orchids have published 13 new species belonging to various genera and one new genus (*Neo-urbania*); the new genus is based on

Ponera adendrobium Reichb.—J. A. PURPUS (Monats. Kateenk. **19**:52, 53, 89. 1909) describes and illustrates two new species of Cereus from Guatemala.—F. EICHLAM (*ibid.* 59, 60) has published a new variety of *Mamillaria Celsiana* Lem. from Guatemala.—T. S. BRANDEGEE (Univ. Cal. Pub. Bot. **3**:377–396. 1909), under the title "Plantae mexicanae Purpusianae," has published 43 species and one variety of angiospermous plants as new to science, and proposes the following new genera: *Setchellanthus* of the Capparidaceae, *Acanthothamnus* of the Celastraceae, and *Dichondropsis* of the Convolvulaceae.—H. D. HOUSE (Muhlenbergia **5**:65–72. 1909) has described 7 new species of American Convolvulaceae and made several new combinations.—B. SCHROEDER (Ber. Deutsch. Bot. Gesells. **27**:210–214. 1909), under the title of "Phytoplankton von Westindien," lists 71 species from West Indian waters, including one new to science.—J. BRIQUET (Ann. Conserv. et Jard. Bot. Genève **11–12**:175–193. 1908) has published 10 new species and 4 new varieties of Ranunculus and Geranium from Mexico and South America.—N. WILLE (Nyt. Mag. Naturv. **47**:— (reprint 1–21. *pls. 1–4.* 1909) describes and illustrates a new genus (*Wittrockiella*) of the Chaetophorales, represented by a single species, *W. paradoxa*. The material on which the new genus is based was collected near Lyngor on the southeast coast of Norway. The author proposes for it the new family *Wittrockiellaceae* and states that its nearest affinity is with the Chroolepidaceae.—E. HASSLER (Bull. Soc. Bot. Genève II. **1**:207–212. 1909) proposes a new genus (*Pseudobastardia*) of the Malvaceae from South America.—H. CHRIST (*ibid.* 216–236) in continuation of his treatment of the ferns of Costa Rica, for the "Primitiae florae costaricensis," has published 27 new species and one monotypic new genus (*Costaricia*), also 2 new species of Lycopodium.—E. L. GREENE (Rep. Nov. Sp. **7**:1–6. 1909) publishes 17 new species of the genus Aconitum from western America.—F. KRÄNZLIN (*ibid.* 38–41) has described new species in the Orchidaceae, some of which are from South America, and (*ibid.* 114, 115) a new species of Epidendrum from Mexico.—T. HERZOG (*ibid.* 49–69), in collaboration with different specialists, has published 37 new species of Siphonogamae from South America, chiefly from Bolivia.—A. COGNIAUX (*ibid.* 69–72) has described six new species and varieties of Orchidaceae and Melastomaceae from Paraguay, and (*ibid.* 121–123) four new orchids from Jamaica.—E. HASSLER (*ibid.* 72–78) has published six new species and varieties of Malvaceae and Leguminosae from Paraguay and proposes a new genus (*Pseudopavonia*).—A. LINGELSHEIM, F. PAX, and H. WINKLER (*ibid.* 107–114), under the title "Plantae novae bolivianae," have published 20 new species of flowering plants.—W. BECKER (*ibid.* 123, 124) records two new species of Viola from Peru.—E. PALLA (Oester. Bot. Zeits. **59**:186–194. *pl. 3.* 1909) has published several new Cyperaceae, including a new species of Bulbostylis from Bolivia.—E. ULE (Verh. Bot. Ver. Brand. **50**:69–123. 1909), in cooperation with several specialists, has published 70 new species of flowering plants from South America, based on collections made by himself in the region of the Amazon; two new genera are proposed: *Chamaeanthus* of the Commelinaceae and *Dolichodelphys* of the Rubiaceae.—P. HENNINGS (*ibid.* 129–136) has described several new fungi, including

a new monotypic genus (*Exogone*), which the author refers to the Rhizinaceae; the material on which the new genus is based was found growing on partially decayed leaves of cabbage.—T. MAKINO (Bot. Mag. Tokyo **23**:59–75. 1909) in continuation of his studies on the flora of Japan describes several new species and proposes a new monotypic genus (*Ortherodendron*) of the Celastraceae, based on *Elaeodendron japonicum* Franch. & Sav.—W. SUKATSCHEFF (Jour. Bot. St. Pétersb. **3**:124–136. 1908) gives an account of an alga recently discovered in Lake Lunoevo, Russia, for which the author proposes the generic name *Lunoevia;* illustrations supplement the description.—V. L. KOMAROV (Acta Hort. Petrop. **29**:179–362. *pls. 5–20.* 1909) presents a monographic treatment of the genus Caragana, in which 56 species are recognized, 27 being new to science; the genus has its distribution through central Asia and China.—E. B. COPELAND (Phil. Jour. Sci. **4**:1–64. *pls. 1–21.* 1909), in an article entitled "Ferns of the Malay-Asiatic region, part I," including all families of ferns for the region except Hymenophyllaceae and Polypodiaceae, recognizes 22 genera, to which are referred 196 species. The genus Cyathea dominates, being there represented by 101 species. Each genus is illustrated by reproduced photographs, but from rather fragmentary material.—C. B. ROBINSON (*ibid.* 69–105) records for the Philippine Islands three species of the Chloranthaceae, of which one is new, and some 55 species of the section Phyllanthinae of the Euphorbiaceae, of which 21 are new to science.—R. MUSCHLER (Bot. Jahrb. **43**:1–74. 1909), under the title "Sytematische und pflanzengeographische Gliederung der afrikanischen Senecio-Arten," presents a detailed consideration of the genus, as it pertains to Africa, recognizing about 500 species, 28 of which are here described for the first time.—H. D. HOUSE (Ann. N. Y. Acad. Sci. **18**:181–263. 1908) presents a monographic treatment of the North American species of Ipomoea, in which 175 species and several varieties are recognized, 30 being new. The author gives concise keys, rather full synonomy, and numerous citations of well-known series of exsiccatae, thus making a very useful synopsis for the identification of material of this group. The revision excludes Operculina, Quamoclit, Exogonium, Calonyction, Turbina, and Rivea.—P. C. STANDLEY (Muhlenbergia **5**:81–87. 1909) has described 5 new species of Castilleja from the southwest.—E. O. WOOTEN and P. C. STANDLEY (*ibid.* 87) describe a new species of Lathyrus from New Mexico.—J. M. GREENMAN.

Cytology of a Drosera hybrid.—Since the preliminary announcement of ROSENBERG'S work on the hybrid *Drosera longifolia*×*rotundifolia*, cytologists have awaited with some impatience the more detailed account, which has now appeared.[3] Besides giving a comparative study of the external features of the two parents and their hybrid, the present paper describes the behavior of the chromosomes in critical phases of the life-history of both parents, and gives an extended account of the chromosomes of the hybrid.

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[3] ROSENBERG, O., Cytologische und morphologische Studien über *Drosera longifolia*×*rotundifolia*. Kungl. Svenska Vetenskapsakad. Handl. **43**:1–64. *pls. 1–4.* 1909.

In *D. longifolia* there are 40 chromosomes in the nuclei of the sporophyte and 20 in those of the gametophyte, while in *D. rotundifolia* the numbers are 20 and 10 respectively. The chromosomes of *D. rotundifolia* are somewhat smaller, as well as less numerous. The behavior of the chromatin in a hybrid between two such forms is naturally of some importance.

In the hybrid, called *D. obovata*, the nuclei of the sporophyte show regularly 30 chromosomes, the anticipated number, but in the nuclei of spore mother cells the condition is unique. At the metaphase of the heterotypic mitosis in the pollen mother cell there appear 10 double chromosomes, presumably resulting from the pairing of 10 chromosomes of *D. longifolia* with the 10 of *D. rotundifolia*. Besides, there are 10 smaller single chromosomes, presumably belonging to *D. longifolia*. These 10 smaller chromosomes are irregularly distributed; some enter the daughter nucleus at the close of this mitosis, while others remain in the cytoplasm and may organize small nuclei, as in the well-known case of Hemerocallis. The behavior at the second mitosis is similar. The four spores of the pollen tetrad stick together, so that it is possible to determine the entire number of chromosomes in the four nuclei. Counting the chromosomes in the four nuclei and including those of the dwarf nuclei, the number is about 60. In any given spore the number ranges from 10 to 15, with 14 the most frequent. In a preliminary paper, ROSENBERG concluded that two of the spores of a tetrad belonged to *D. longifolia* and two to *D. rotundifolia*. This conclusion is now withdrawn, and differences in the size of spores is attributed to differences in the number of chromosomes. Sometimes a generative cell is formed, but usually the contents of the spore begin to disorganize before this stage is reached. At the time of shedding, the pollen grain has a normal exine, but the contents are usually dead.

In the formation of four megaspores from the mother cell the behavior is very similar to that just described. Occasionally, there is a well-developed embryo sac, but in most cases disorganization begins before the four-nucleate stage is reached.

ROSENBERG crossed the hybrid *D. obovata* with *D. longifolia*, and while usually there was no result, he obtained a few embryos. These contained at least 33 chromosomes, and in one case 37 were counted. The theoretical number would be 35.

The principal conclusions are (1) that the chromosome is an individual organ of the cell, and (2) reduction of chromosomes is brought about by a fusion of the chromosomes of the two parents.—CHARLES J. CHAMBERLAIN.

Mildew on Alchemilla species.—STEINER[4] has published an interesting paper on the specialization of *Sphaerotheca Humuli* (DC.) Burr on various species of Alchemilla. In addition to finding that the mildew on Alchemilla is confined to species of this genus, he also claims to be able to distinguish "minor biological species" within this genus of host plants. For example, conidia

[4] STEINER, J. A., Die Spezialization der Alchemillen-bewohnenden *Sphaerotheca Humuli* (DC.) Burr. Centralbl. f. Bakt. etc. **21²**:677–726. 1908.

from *A. pastoralis* and *A. flexicaulis* are alike in infecting capacity, except that conidia from the former will only partially infect *A. pubescens*, and not *A. alpigena* at all; while conidia from *A. flexicaulis* partially infect *A. alpigena*, *A. pubescens* being entirely immune. Another case is that of the mildew on *A. impexa* which does not infect *A. alpina vera* or *A. nitida*, while conidia from *A. pastoralis* partially infect these hosts. Otherwise the two mildews are alike. STEINER further found that conidia from species of the *Vulgares* group will not produce full infection on alpine species, although conidia from alpine species produce full infection on the *Vulgares* species. STEINER supposes that the mildew on the alpine species came originally from *Vulgares* species and is only partially adapted to the new hosts. He also believes that the appearance of the mildew on alpine species is due to unfavorable environment.

STEINER also claims to have found "bridging species;" for example, conidia from *A. nitida* infect *A. impexa* but not *A. fallax*, while conidia from *A. impexa* will infect *A. fallax*. Thus the mildew is carried over from *A. nitida* to *A. fallax* through *A. impexa*. Similarly, *A. pastoralis* and *A. impexa* transfer the mildew from *A. connivens* and *A. pubescens* to *A. micans*. In addition to the fact that only a few tests were made, STEINER does not tell us what are the infecting powers of the mildews produced in this way on *A. micans* and *A. fallax*.

His conclusions would be more convincing if based on a larger number of tests. A large number of foreign infections also occurred in his experiments, no less than 71 foreign infections occurring in a total of 380 tests. The results are presented very clearly by means of a series of well-devised diagrams.—GEORGE M. REED.

Cytology of Florideae.—Cytological studies on the Florideae have been comparatively rare, partly on account of the difficulty in securing material, but principally on account of the difficult technic. Quite recently KURSSANOW has published[5] the results of his studies on three different forms of red algae: *Helminthora divaricata*, *Nemalion lubricum*, and *Helminthocladia purpurea*. His investigations did not deal with nuclear details, but rather with the morphology of fertilization of the carpogonium, the development of carpospores, and the structure of the chromatophores.

He failed to find a nucleus in the trichogyne of Nemalion and Helminthora; the trichogyne in these forms seems to be an extension of the carpogonium. He believes that such a condition is found only in the simplest forms of red algae, and agrees with the reviewer that a trichogyne with a nucleus, and yet without a partition wall between it and a carpogonium, as in Polysiphonia, may be a forerunner of the multicellular trichogyne found in the Laboulbeniaceae. The spermatium (sperm) has a single nucleus, agreeing with the reviewer's description of Polysiphonia. He thinks that a uninuclear condition in the sperm may perhaps be universal in red algae. In Nemalion, contrary to WOLFE's results, the chromatophore has, in its center, a well-formed pyrenoid which is composed

[5] KURSSANOW, L., Beiträge zur Cytologie der Florideen. Flora **99**:311–336. *pls. 2, 3.* 1909.

of two parts, a central portion and the surrounding zone. The pyrenoid is influenced by its environment, and easily becomes swollen and dissolved, leaving vacuoles in its place. Such a compound structure of the pyrenoid is shown only in the stained preparation, and when it is not differentiated with stains the pyrenoid appears quite homogeneous. SCHMITZ's description of the pyrenoid as a homogeneous body may perhaps be based upon the unstained material.—SHIGÉO YAMANOUCHI.

Karyokinesis in Oedogonium.—Since STRASBURGER's and KLEBAHN's work on Oedogonium, there had been little published on mitosis in this form until WISSELINGH's paper appeared. STRASBURGER's material was *O. tumidulum* Kg., KLEBAHN's *O. Boscii* Witte, and WISSELINGH's material was *O. cyathigerum* Witte,[6] fixed in Flemming's solution. After being left in the solution for one day, it was treated with 20 per cent. chromic acid. By the action of the Flemming solution and the chromic acid solution, the cell wall and cell contents become entirely dissolved, and the nuclear membrane is also dissolved by the action of 20 per cent. chromic acid solution. The chromosomes during mitosis were studied in their isolated condition.

The chief points of interest are as follows: The mitosis in Oedogonium agrees with that of higher plants; the development of chromosomes out of the nuclear network, the formation of the nuclear plate, the longitudinal splitting of the chromosomes, the reconstruction of daughter nuclei seem like these processes in Fritillaria and Leucojum, two forms which were also studied by VON WISSELINGH. In Oedogonium, the chromosomes, 19 in number, and differing greatly from one another in length, are connected by fine fibrils. The nucleolus does not take part in forming chromosomes, but disappears at the beginning of mitosis, and there appear in daughter nuclei new nucleoli, which later unite into one.—SHIGÉO YAMANOUCHI.

Mycorhiza.—PEKLO announces in a preliminary paper[7] the results of his studies on the epiphytic mycorhiza of Carpinus and Fagus, with brief reference also to the endophytes of *Alnus glutinosa* and *Myrica Gale*.

In Carpinus, as a reaction to the penetration of the tissues of the young rootlet, tannins increase (as the author has also determined for Monotropa[8]), and this restricts the fungus to the intercellular spaces. Nourishing itself partly on this glucoside and other foods in the cortex, the fungus forms the jacket, the outermost hyphae of which often die. Isolation of the fungus was finally accomplished by using a decoction of old thick mycorhizas, which proved very specific for the

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[6] WISSELINGH, C. VON, Ueber die Karyokinese bei Oedogonium. Beih. Bot. Centralbl. **23**:139–156. *pl. 7.* 1908.

[7] PEKLO, J., Beiträge zur Lösung des Mykorhiza-Problems. Ber. Deutsch. Bot. Gesells. **27**:239–247. 1909.

[8] ———, Die epiphytischen Mykorhizen nach neuen Untersuchungen. I. *Monotropa Hypopitys* L. Bull. Böhm. Akad. Wiss. 00:000. 1908.

infecting fungus. In this the inner hyphae began to grow and broke through the outer layers, and on this mycelium, whose origin was clear, conidiophores and conidia arose within three days. These showed it to be a Penicillium (Citromyces) very like *P. geophilum*, and similar results were reached with Fagus. Fungi of this group were also found in the forest soil where mycorhiza of Fagus was abundant. Carpinus was not available for experiments on reinfection, but a considerable number of young roots of a two-year-old Fagus showed infection from pure cultures of the Carpinus mycorhiza, as well as from several other species of forest Penicillia.—C. R. B.

Respiration.—For about a dozen plants Mme. MAIGE has determined the amount of O_2 fixed and CO_2 evolved by the stamens and pistils as compared with an equal weight of leaf tissue, both in air and in pure hydrogen.[9] She finds both aerobic and anaerobic respiration, tested thus, to be much (2–18 times) more active in the floral organs than in the leaf; and, with one exception, more vigorous in the pistil than in the stamen, and in the anther than in the filament. These results confirm the early ones (1822) of DE SAUSSURE, as to the relative rate of respiration of the floral organs and the leaves; but DE SAUSSURE found stamens more active than pistils. For the conciseness of this paper Mme. MAIGE is much to be commended.

JENSEN[10] finds that the alcoholic fermentation of sugar proceeds by two stages and he therefore predicates two enzymes, glucose being split by dextrase (glucase ?) into dioxyacetone and this by "dioxyacetonase" into CO_2 and alcohol. But in respiration, with oxidase and free oxygen present, the dioxyacetone, produced as in fermentation, breaks up into CO_2 and water, the main end-products of aerobic respiration.—C. R. B.

Transpiration.—SAMPSON and ALLEN, declaring that too little account has been taken of the effect of physical factors on transpiration, furnish further data on this subject.[11] Comparing evaporation from equal areas in equal times they find that there is little variation for plants of the same species under the same conditions of development and exposure; that of the same species the sun form evaporates 2–4 times as much as the shade form, whether the two are tested in the sun or shade, a difference which they ascribe chiefly to the greater number of stomata in the sun form (20–60 per cent.); that the increased evaporation with altitude, *caeteris paribus*, is due to lower pressure and not to differences in light or humidity; that generally acid solutions accelerate and alkaline solutions retard evaporation, but without relation to concentration; that evaporation is greater

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[9] MAIGE, MME. G., Recherches sur la respiration de l'étamine et du pistil. Rev. Gén. Bot. **21**:32–38. 1909.

[10] JENSEN, P. BOYSEN, Die Zersetzung des Zuckers während des Respirationsprozesses. Ber. Deutsch. Bot. Gesells. **26a**:666, 667. 1908.

[11] SAMPSON, A. W., AND ALLEN, LOUISE M. Influence of physical factors on transpiration. Minn. Bot. Stud. **4**:33–59. 1909.

from plants in coarse than in fine soils; and that a "bog xerophyte," *Scirpus lacustris*, loses about twice as much water as *Helianthus annuus*, on account of its loose structure, the air spaces being estimated at 80 per cent. of the total volume and the internal surface as 15 times the external.—C. R. B.

Anthocyan.—On the vexed question of the formation of anthocyan, COMBES furnishes[12] first a very clear and compact summary of the previous researches. He then demonstrated that the close relations between the accumulation of carbohydrates and the formation of anthocyan, pointed out by the researches of OVERTON and MOLLIARD on artificially nourished plants, exist also in nature, however the pigmentation is provoked. The insoluble carbohydrates behave differently, according to the occasion of the pigmentation; but the sugars, glucosides, and dextrins behave alike in all cases, the two former varying in amount directly as the anthocyan, the dextrins diminishing as the sugars and glucosides increase. The insoluble carbohydrates, consequently, appear not to share directly in the formation of the red pigment. COMBES concludes that the anthocyans, which are probably cyclic glucosides, are formed at the expense of neither preexistent sugars and glucosides nor chromogens, but arise at the same time as other glucosides, as part of the general accumulation of such bodies.—C. R. B.

Chlorophyll bodies.—Morphological distinctions between chlorophyll bodies, found in a great number and variety of plants, have been pointed out by D'ARBAUMONT,[13] who divides them into two categories, chloroplasts and pseudochloroplasts. The former, held to be morphologically superior, seem to include the bodies usually recognized under that name, without admixture of the latter, from which they are distinguished by not swelling in water (at least *in situ*), and by not being stained, with rare exceptions, by acid aniline blue. The pseudochloroplasts, on the contrary, usually swell in water and become vividly colored in the stain. They are of four types, all small, more or less varied in shape, with different degrees of green coloration, and variously intermixed. The members of the two categories are formed in the same way, either with or without the cooperation of starch,[14] and both, without reference to their mode of origin, may or may not form starch.—C. R. B.

Morphology of Symplocarpus.—In an investigation of *Symplocarpus foetidus*, ROSENDAHL[15] has obtained the following results: the primordia of the flowers

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[12] COMBES, R., Rapports entre les composés hydrocarbonés et la formation de l'anthocyane. Ann. Sci. Nat. Bot. IX. **9**: 275–303. 1909.

[13] D'ARBAUMONT, J., Nouvelle contribution a l'étude des corps chlorophylliens. Ann. Sci. Nat. Bot. IX. **9**: 197–229. 1909.

[14] *Cf.* BELZUNG, E., Nouvelles recherches sur l'origine des grains d'amidon et des grains de chlorophylle. Ann. Sci. Nat. Bot. VII. **13**: 17. 1891; Jour. de Bot. **9**: 67, 102. 1895.

[15] ROSENDAHL, C. OTTO, Embryo sac development and embryology of *Symplocarpus foetidus*. Minn. Bot. Studies **4**[1]: 1–9. *pls. 1–3*. 1909.

appear 18–20 months before the pollination period, and the ovules are formed late during the season preceding pollination; the single archesporial cell produces four distinct megaspores; an antipodal tissue of a considerable number of cells with large nuclei is developed; endosperm formation begins with free nuclear division, and this is followed by a walled-tissue which fills the embryo sac and encroaches upon the integuments and the chalaza; a filamentous proembryo (2 or 4 cells) becomes club-shaped to ovoid, and a short suspensor of several rows of cells is differentiated from the usual monocotyledonous embryo; in its growth the embryo completely destroys the endosperm and all other ovular structures, and comes to lie naked in the cavity of the ovary, so that there are no seeds in the ordinary sense.—J. M. C.

Morphology of Caulophyllum.—The seed and seedling of *Caulophyllum thalictroides* have been studied by BUTTERS,[16] with the following results: the fleshy testa incloses a very hard endosperm, which has almost completely destroyed the inner integument; the proembryo is massive and pear-shaped and the cotyledons appear late; the first season's growth after germination is usually entirely subterranean, the cotyledons together forming an effective haustorium; the first leaves are usually scalelike and inclose a winter bud; each cotyledon sends three vascular bundles into the hypocotyl, which finally form a tetrarch root; secondary thickening takes place in the hypocotyl, resulting in the formation of a continuous zone of xylem about the pith.—J. M. C.

Temperature and locomotion.—TEODORESCO reports[17] movements in certain organisms at temperatures far lower than have heretofore been recorded. Thus he found zoospores of Dunaliella motile down to temperatures of $-17°$ to $-22°.5$ C., and others at $-5°$ to $-12°.7$ C. The limits vary with species and even with individuals. There seems to be much more activity in winter, even among freshwater organisms, than has been supposed.—C. R. B.

Carbon monoxid.—KRASCHÉNNIKOFF, after a careful series of experiments, reports[18] that CO cannot be used by green plants to form carbohydrate. The view of BOTTOMLEY AND JACKSON,[19] which was really not adequately supported by their experiments, the only ones interpreted in favor of such use, is distinctly negatived.—C. R. B.

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[6] BUTTERS, FREDERIC K., The seeds and seedling of *Caulophyllum thalictroides*. Minn. Bot. Studies **4**[1]:11–32. *pls. 4–10.* 1909.

[7] TEODORESCO, E. C., Recherches sur les mouvements de locomotion der organismes inférieurs aux basses températures. Ann. Sci. Nat. Bot. IX. **9**:231–274. 1909.

[18] KRASCHÉNNIKOFF T., La plante verte assimile-t-elle l'oxyde de carbone? Rev. Gén. Bot. **21**:177–193. *pl. 10.* 1909.

[19] Proc. Roy. Soc. Lond. **72**:130–131. 1903.

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October 1909

Editors: JOHN M. COULTER and CHARLES R. BARNES

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BOTANICAL GAZETTE

OCTOBER 1909

THE EMBRYO SAC OF HABENARIA[1]

WILLIAM H. BROWN

(WITH TWELVE FIGURES)

The present investigation was made on *Habenaria ciliaris* (Michx.) R. Br. and *H. integra* (Nutt.) Spreng. The development of the two species is very much alike and the same description will apply to both. The material was fixed in medium chromacetic and the sections were

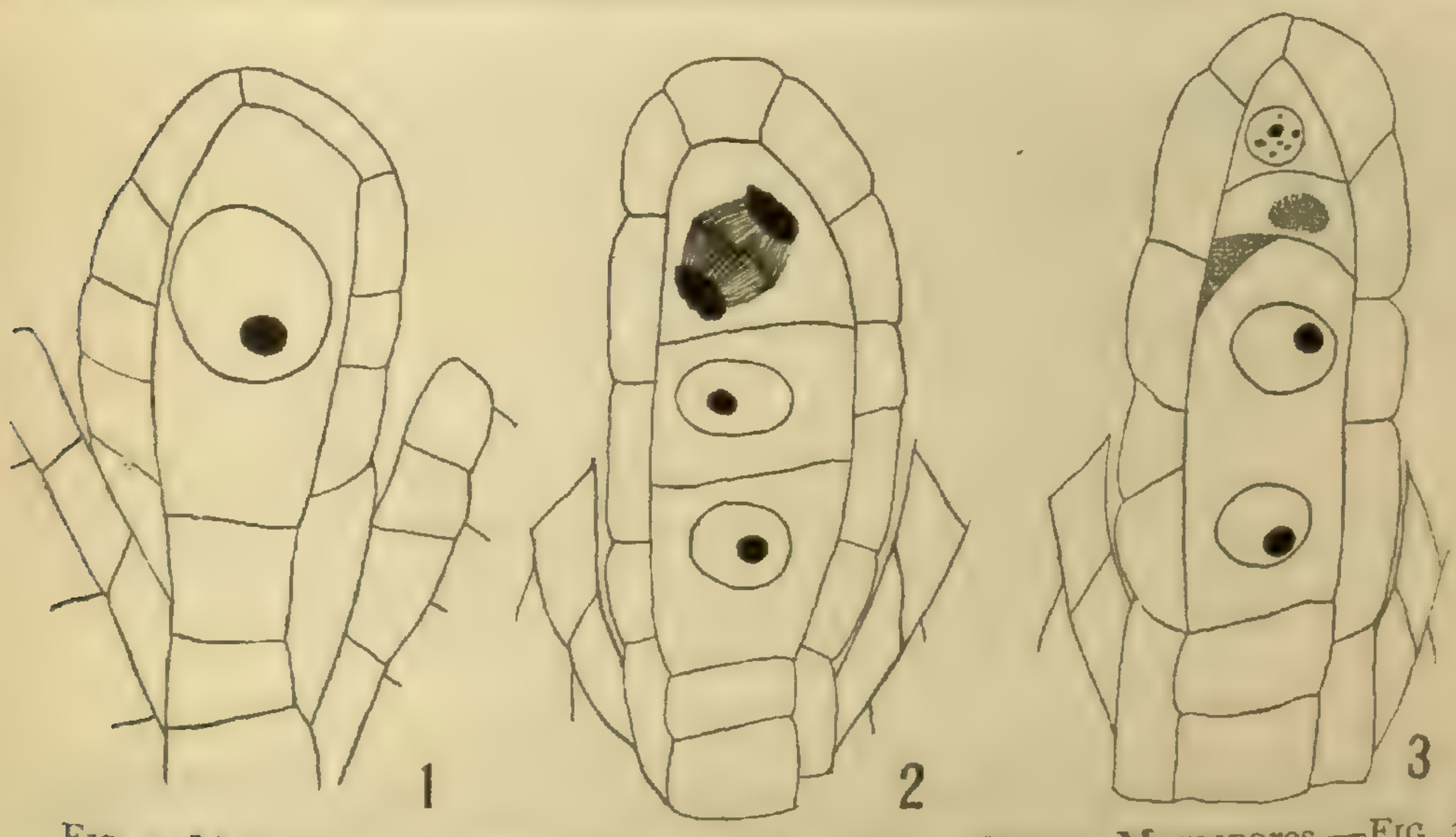


FIG. 1. Megaspore mother cell in apex of nucellus.—FIG. 2. Megaspores.—FIG. 3. First division of functional megaspore and three degenerating megaspores.

cut 10 μ thick and stained with Flemming's triple or Haidenhain's iron alum hematoxylin.

The ovule of Habenaria is anatropous and has two integuments. The archesporium differentiates in the apex of the nucellus as a single hypodermal cell (*fig.* 1), which is the terminal cell of a row surrounded

[1] Contribution from the Botanical Laboratory of the Johns Hopkins University, No. 10.

only by the epidermal layer. The archesporial cell without dividing functions as a megaspore mother cell, which divides to two daughter cells, which in turn divide to form four megaspores. The last division may be simultaneous in both daughter cells, but usually it is delayed in the one nearest the micropyle (*fig. 2*).

Soon after the formation of the megaspores the chalazal one begins to enlarge at the expense of the other three, which soon degenerate. Before this degeneration has proceeded far the nucleus of the functioning megaspore divides (*fig. 3*) and the two daughter nuclei remain

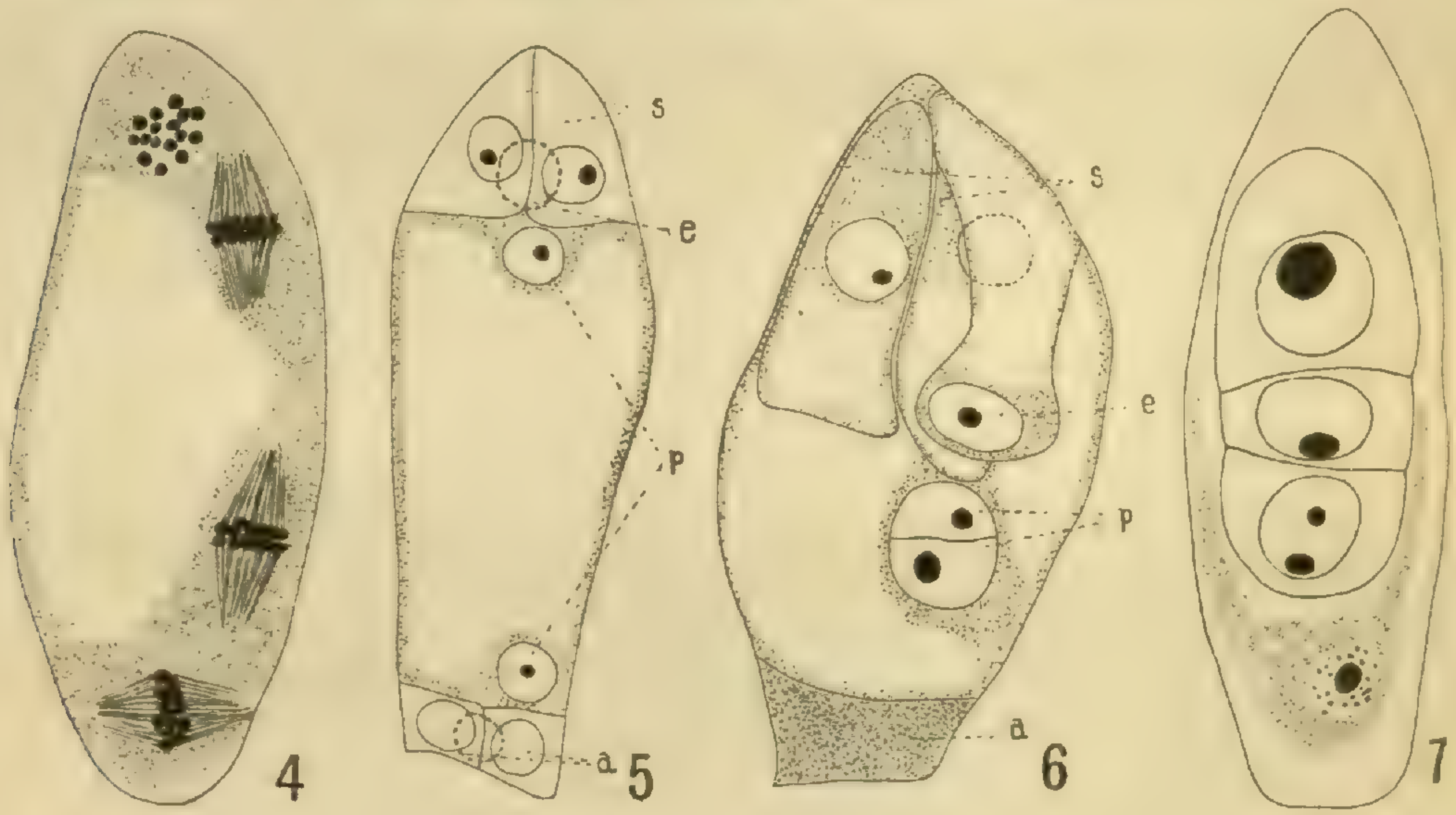
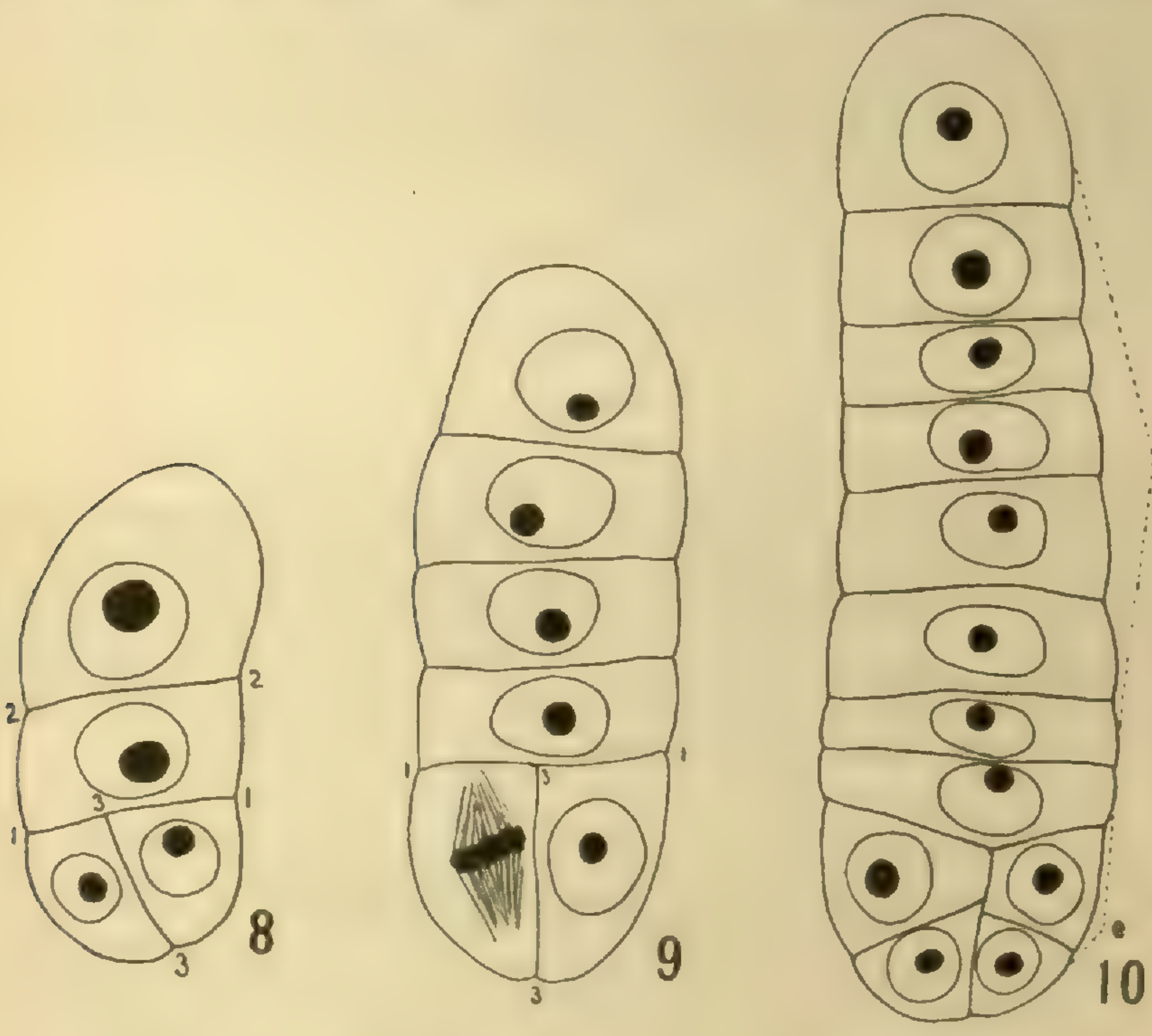


FIG. 4. Nuclei of four-nucleate embryo sac dividing to eight.—FIG. 5. Eight-nucleate embryo sac: *s*, synergids; *e*, egg; *a*, antipodals; *p*, polar nuclei.—FIG. 6. Embryo sac with fusing polar nuclei; *p*, polar nuclei; *s*, synergids; *e*, egg; *a*, degenerating antipodals.—FIG. 7. Young embryo and degenerating endosperm nucleus.

in the polar positions in the enlarging sac. Each of these two nuclei by two successive divisions gives rise to four, so that the mature sac contains eight nuclei (*fig. 5*). As the sac continues to enlarge, the nucellar cells which surrounded the four megaspores degenerate, so that at about the four-nucleate stage the sac comes to lie against the inner integument. At the last division of the embryo sac nuclei the two spindles in each end are arranged approximately at right angles to one another (*fig. 4*). As is usual, the transverse spindle in the micropylar end gives rise to two synergids, while the longitudinal one forms the egg and the micropylar polar nucleus. In the chalazal end the longitudinal spindle gives rise to the antipodal polar nucleus and one

antipodal, while the other spindle forms two antipodals. Walls cutting off the egg, synergids, and antipodals are formed on fibers connecting the nuclei. Owing to their position nearer the center of the sac, the polar nuclei are left free in the cytoplasm (*fig. 5*), as has been pointed out by STRASBURGER (**'05**) for *Drimys Winteri*. After their formation, the egg and synergids enlarge considerably, while the antipodals soon degenerate (*fig. 6*). When the egg and synergids have about reached their mature size, the polar nuclei fuse (*fig. 6*). No cases were observed in which the polar nuclei were in con-



FIGS. 8–10. Older embryos: *s*, suspensor; *e*, embryo.

tact without fusing, as have been described for other forms by STRASBURGER (**'00**) and NAWASCHIN (**'00**). The pollen tube comes through the micropyle and discharges the two male nuclei into the embryo sac. One of these fuses with the egg, while the other fuses with the product of the fusion of the polar nuclei to form the primary endosperm nucleus. This enlarges somewhat, but without dividing begins to degenerate at about the time of the first division of the fertilized egg (*fig. 7*).

The first division of the fertilized egg is transverse to the longitudinal axis of the embryo sac. Of the two resulting cells the chalazal one

forms most of the embryo, while the micropylar one gives rise to the suspensor and a small part of the young embryo. The second division (*fig. 8, wall 2*) is in the micropylar cell and is also transverse; the third division (*fig. 8, wall 3*) is in the chalazal cell and is longitudinal. Divisions continue in the descendants of the micropylar cell until a row of about eight cells is formed (*fig. 10*). The seven of these which are nearest the micropyle make up the suspensor, while later on the other one divides to form part of the embryo (*fig. 11*). The first division in this cell may be either transverse or longitudinal. When the row of cells derived from the primary micropylar cell has become four or more cells long, transverse segmentation begins in the descendants of the chalazal cell (*fig. 9*), so that this becomes cut into quadrants (*fig. 10*). Up to this stage the embryo and suspensor have remained within the embryo sac cavity, but now the cells of the suspensor elongate and the free end of the suspensor is pushed through the micropyle out beyond the integuments (cf. shape of cells in *figs. 10* and *11*). The cells of the embryo undergo further division and form a globular mass; *fig. 12* represents a young embryo in which *walls 1* and *3* correspond to *1* and *3* of *figs. 8–11*.

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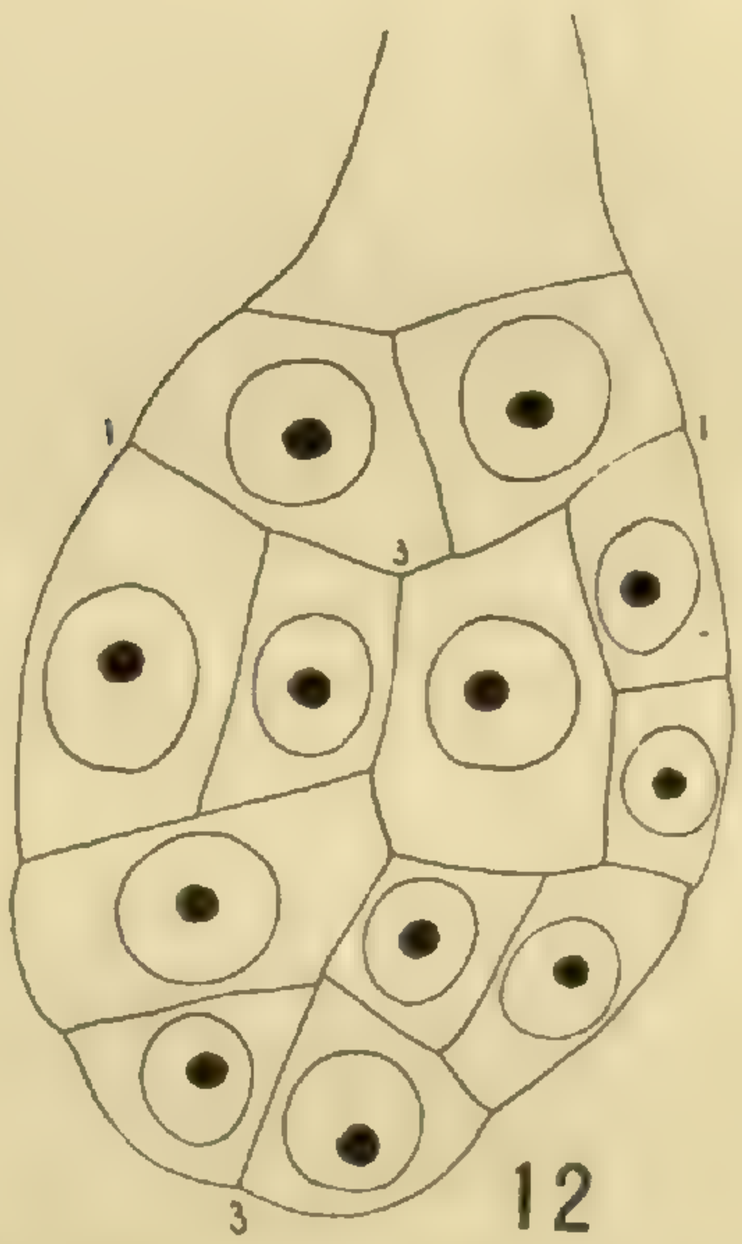

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FIG. 11. Still older embryo drawn on a smaller scale; the suspensor has elongated. —FIG. 12. Many-celled embryo.

Discussion

MEGASPORES

In Habenaria the megaspore mother cell divides to two daughter cells and each of these divides to two megaspores. The division in the daughter cell nearest the micropyle is usually delayed until after that in the daughter cell nearest the chalazal end. If this delay was carried a little farther, we should have the condition described in

several other orchids (WARD **'80,** STRASBURGER **'84**), which have only three megaspores.

In Cypripedium (PACE **'08**) the daughter cells of the megaspore mother cell do not divide, but one of them forms the embryo sac. In this case the question arises as to whether the division of both daughter cells has been omitted, in the way indicated for one of them in Habenaria, and the place of the second "reducing division" changed to the embryo sac mother cell (the cell within the walls of which the embryo sac is organized); or whether the first two nuclei of the embryo sac are, as Miss PACE calls them, megaspore nuclei. In favor of the latter view it may be said that the "completion of chromosome reduction" which takes place in the division of the daughter cell is necessary to the normal development of the embryo sac. COULTER (**'08**) thinks that because chromosome reduction, which is usually associated with megaspore formation, is necessary that megaspore nuclei cannot be omitted. On the other hand, the place of reduction is not always constant, even in nearly related plants, as in the case of Nemalion (WOLFE **'04**) and Polysiphonia (YAMANOUCHI **'05**). In Nemalion the fertilized egg divides to carpospores and, according to WOLFE, reduction takes place in their formation. In Polysiphonia reduction does not take place in carpospore formation, but the carpospore germinates to a plant with the diploid number of chromosomes. This plant bears not carpospores but tetraspores, and reduction takes place in the division of the tetraspore mother cell. In speaking of Polysiphonia, YAMANOUCHI says: "The tetrasporic plants may have arisen by a suppression of the reduction phenomena in connection with the carpospore, so that it germinates with the sporophytic number of chromosomes. . . . . The period of chromosome reduction would be thus postponed from the carpospore to a later period in connection with the newly formed plant." If, as seems probable, the place of reduction has been changed among the red algae, it is reasonable to suppose that it may also have been changed in some angiosperms, and especially if the structure in which it normally occurred had been left out of the life-history of the plant. The leaving-out of the division of the the mother cell into megaspores would simply be the completion of a tendency toward the reduction in the number of divisions of the archesporial cells from the condition in the ferns (where it divides a number

of times) to such a condition as Habenaria where the archesporial cell without dividing functions as a spore mother cell.

The only way in which we can claim that megaspore nuclei must accompany chromosome reduction is by defining a spore mother cell as that cell in which reduction is initiated and spore nuclei as the nuclei resulting from the reducing divisions. The logical conclusion of this would be to make chromosome behavior the sole criterion for distinguishing spores, sporophytes, and gametophytes; but since the four megaspores and embryo sac of Alchemilla (MURBECK '01) may have the diploid number of chromosomes, or the apogamous embryos of Nephrodium (YAMANOUCHI '08) the haploid number of chromosomes, we cannot regard chromosome behavior as the sole, if indeed the most important, criterion for distinguishing any of these structures.

A distinction between the first division of the megaspore and a division giving rise to megaspores is that while in the first case no cell plate is formed on the spindle, in the latter case either a wall or a cell plate is formed on the spindle. This wall may be formed in the embryo sac when this is derived from more than one megaspore, as is apparently the case in Peperomia (BROWN '08). The first division of the embryo sac mother cell nucleus of Cypripedium (PACE '08) agrees with that of those derived from one megaspore in having no cell plate formed on the spindle.

The evidence for either view is inconclusive, but seems to the writer to be in favor of the idea that in Cypripedium the second division in megaspore formation has been left out, and the place where "reduction is completed" changed to the first division of the nucleus of the embryo sac mother cell.

LLOYD ('02) and COULTER ('08) have advanced the idea that when megaspores are not formed the first four nuclei of the embryo sac are megaspore nuclei. This is probably true in such cases as Lilium (COULTER and CHAMBERLAIN '03) and Peperomia (BROWN '08), and as COULTER ('08) suggests in some other sixteen-nucleate embryo sacs; but if the ideas brought forward here concerning Cypripedium are correct, it need not be of universal application. CAMPBELL ('09), however, seems to think that even the embryo sacs of Lilium and Peperomia are not derived from more than one megaspore.

In discussing the view of COULTER ('08) that when the megaspore

mother cell does not divide the first four nuclei of the embryo sac are megaspore nuclei, and of BROWN ('08) that the embryo sac of Peperomia is composed of the descendants of four megaspores, CAMPBELL ('09) says: "The generally accepted view that in such cases as Peperomia and Lilium the embryo sac is a single megaspore formed without previous division from the mother cell can hardly be admitted to have been disproved by these recent speculations." In speaking of Peperomia, he says that BROWN bases his opinion that the first four nuclei of the embryo sac represent megaspores upon the fact that cell walls are formed in the first two nuclear divisions in *Peperomia sintensii* and cell plates in the same divisions in *P. pellucida;* while in the third division cell plates are wanting. CAMPBELL says that since in the last division, which gives rise to sixteen nuclei, cell walls are formed, "this seems rather inadequate grounds for assuming that the embryo sac represents four spores rather than a single one." The presence of the cell walls was not the only reason for thinking that the embryo sac of Peperomia represents four spores; but even if it were, the formation of walls in the last division would offer no difficulty to such a view, for walls are usually formed at the last division of the embryo sac of angiosperms and the writer has not been able to find any essential difference between the method of their formation in Peperomia and in Habenaria. Nor would the reduction of the free divisions in the embryo sac to a single one (the third in Peperomia) be a difficulty when we remember that the number of these is often large but quite variable in the gymnosperms and has been reduced to two in the ordinary angiosperms. The fact remains that unless we assume that the first four nuclei of the embryo sac of Peperomia and Lilium are megaspore nuclei, we have no explanation for the presence of walls in the first two divisions of the embryo sac of Peperomia and for the absence of these walls in the third division, or for the presence of cell plates on the spindles of the first two divisions of Lilium, features which have been described in no case, so far as the writer knows, in which the embryo sac is formed from one of four megaspores.

CAMPBELL thinks that if the compound nature of the sac of Lilium be admitted, we still have to explain the extraordinary departure of Peperomia from the usual type, but why two such distantly related plants would be expected to behave alike is not explained.

It does not seem likely that a primitive embryo sac, as CAMPBELL believes the sixteen-nucleate type to be, has been retained in plants so far examined only in such distantly related genera as Peperomia, Pandanus (CAMPBELL '09), Gunnera (ERNST '08), Euphorbia (MODILEWSKI '09), and the Penaeaceae (STEPHENS '08); and especially since most of these genera are anything but primitive in other respects, and the embryo sac is in most cases derived from a spore mother cell which, as CAMPBELL says, can hardly be regarded as a primitive feature.

ENDOSPERM

NAWASCHIN ('00) finds that in Phajus and Arundina there is no fusion of the polar or second male nuclei, and he attributes the lack of endosperm to this cause. STRASBURGER ('00) has shown that in several European orchids this fusion may or may not take place, but in either case there is no division to form endosperm. He concludes from this that the lack of endosperm is not due to the failure of the nuclei to fuse. The condition in Habenaria, where there is no endosperm, although the fusion nucleus is of constant occurrence, is a confirmation of this view.

In contrast to Habenaria, endosperm may be formed in Lemna (CALDWELL '99) without a fusion of the polar nuclei.

In the aposporous embryo sac of Hieracium (ROSENBERG '06) polar nuclei with the diploid number of chromosomes may fuse to form the endosperm nucleus. In all known sixteen-nucleate sacs all of the nuclei not cut off by walls fuse to form the primary endosperm nucleus. In *Peperomia hispidula* (JOHNSON '07) there are fourteen of these fusing nuclei; while in *P. pellucida* (JOHNSON '00) and *P. sintensii* (BROWN '08) there are eight; in the Penaeaceae (STEPHENS '08) and *Euphorbia procera* (MODILEWSKI '09) there are four; and in Gunnera (ERNST '09) seven.

The fact that in Habenaria the fusion of the polar and second male nuclei does not result in the formation of endosperm, while in Lemna endosperm is formed without this fusion, taken together with the fact that the primary endosperm nucleus may be formed by the fusion of a variable number of nuclei or of nuclei with either the diploid or haploid number of chromosomes, seems to strengthen STRASBURGER'S ('05) view that the endosperm is not a sexually produced embryo, but

that the fusion of the nuclei is connected with the fact that the nuclei have ceased developing and are in the same cell cavity.

Summary

The archesporium of Habenaria arises as a single hypodermal cell, which without dividing functions as a megaspore mother cell.

The mother cell divides to two daughter cells and each of these to two megaspores. The division of the daughter cell nearest the micropyle is usually delayed.

In some cases an embryo sac is probably formed from more than one megaspore; but the condition in the orchids, where there is a gradual reduction of the divisions of the megaspore mother cell without an indication of walls in the embryo sac, indicates that megaspore formation may be omitted and the place of reduction changed to the first division of the nucleus of the embryo sac mother cell.

The embryo sac of Habenaria contains eight nuclei: an egg, two synergids, two polar nuclei, and three ephemeral antipodals.

The primary endosperm nucleus is formed by the fusion of the polar and second male nuclei, but degenerates without dividing.

The absence of endosperm in many orchids offers no support to the view that the endosperm is a sexually produced embryo.

The fertilized egg gives rise to a long suspensor and a globular embryo.

My thanks are due to Professor D. S. JOHNSON for helpful suggestions and criticisms.

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Baltimore, Maryland

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THE INFLUENCE OF TRACTION ON THE FORMATION OF MECHANICAL TISSUE IN STEMS[1]

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Introduction

The following investigation was directed to a further knowledge of the influence of traction in the direction of the longitudinal axis on the formation of mechanical tissue in the stems of plants.

Petioles, tendrils, and roots were not investigated, because the wo k of HEGLER (**9**), BALL (**1**), and HIBBARD (**11**) was primarily on stems, and it was my purpose to add further and more detailed experimental evidence to the work done by these investigators.

This research was carried on in the Botanical Labo atory of the University of Michigan. It was under the direction of Prof. F. C. NEWCOMBE, to whom I am indebted for encouragement and helpful suggestions. I wish, also, to express my appreciation of the interest manifested in my work by the other members of the staff in the botanical department.

Historical

I. STEMS: INFLUENCE OF TRACTION

BARANETSKY (**2**) observed that a stem of *Gesneria tubiflora* weighted with 30^{gm} made less growth than one remaining free. SCHOLTZ (**26**) verified this observation of BARANETSKY, and found that stems when subjected to traction first grew more slowly and later more rapidly than the control plants. This he att ibuted to a change in the physical condition of the cell resulting from the educed hydrostatic pressure. He furnished no experimental evidence to support his theory. HEGLER (**9**) produced the first experimental evidence to show that plants respond to tension by an increased formation of mechanical tissue. This supported the theory which was generally adhered to by plant physiologists previous to that time; viz., that a plant reacts to a gradually increasing strain by an increased

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development of its mechanical tissue. In reporting the work of HEGLER, PFEFFER (**21**) says that a seedling of *Helianthus annuus*, whose original breaking strength was 160^{gm}, had a breaking strength of 250^{gm} after two days under the pull of 150^{gm}. No breaking strength is given for a control. A petiole of *Helleborus niger* which withstood a weight of only 400^{gm}, PFEFFER reports to have held a weight of 3.5^{kg} after 5 days, during which time it had been weighted with a gradually increasing load, while similar petioles under normal conditions gained but little in strength. Changes are also reported in the anatomical structure: (1) an increase in the number of cells of the collenchyma, (2) an increased thickness of the walls of the collenchyma, sclerenchyma, and bast, and (3) the production of entirely new tissues. It is unfortunate that a full account of HEGLER'S methods and work was not published.

HEGLER (**10**) showed that the phenomenon observed by BARANETSKY and SCHOLTZ was not purely physical, but that a physiological change took place within the cell, due to stimulation coming from the tension. As evidence, he demonstrated that when plants were deprived of oxygen or when they were under the influence of chloroform, there was no response. He also found that the plants under tension were more turgid than controls, instead of less as SCHOLTZ had claimed.

RICHTER (**25**) concluded that when the stems of Chara were subjected to a longitudinal pull, there was an increase in strength. BALL'S criticism of RICHTER'S work is well taken. He says: "Die Resultate RICHTER'S sind etwas zweifelhaft, da er keinen Vergleich zwischen belasteten Chara Pflanzen und unbelasteten von demselben Alter und derselben Grösse gegeben hat."

KÜSTER (**15**) found no response, and showed that in the petioles of *Helleborus niger* the new elements, which HEGLER (**91**) claimed were produced in response to tension, were there before tension was applied. PFEFFER (**22,** p. 148) also found no response in the production of mechanical tissue in plants subjected to tension.

VÖCHTING (**27**) investigated the influence of tension on sunflowers and cabbages that had been prevented from flowering by means of decapitation. He found that no new tissue was formed, and that no increase in mechanical tissue occurred as a result of tension.

WIDERSHEIM (**29**) experimented with pendant branches of Fraxinus, Fagus, Caragana, Sorbus, Ulmus, and Corylus. He fastened weights directly to these branches and continued the experiment through most of the growing season. Only in the case of Corylus was there any response; viz., an increase in the number of bast fibers.

BALL (**1**) experimented with *Helianthus annuus*, *Phaseolus multiflorus*, *Lupinus albus*, *Helleborus niger*, *Ricinus communis*, two species of Cyperus, and *Mirabilis jalapa*. His conclusion was that these plants do not respond to tension by an increase of their breaking strength or by the production of mechanical tissue.

HIBBARD (**11**) found an increase of mechanical tissue in the stem of *Vinca major* when the same was subjected to longitudinal pull. He failed to find any response in the stems of *Helianthus annuus*, *Ricinus communis*, *Brassica oleracea*, and *Phaseolus multiflorus*. HIBBARD did not determine the breaking strength of his stems.

2. STEMS: THE INFLUENCE OF COMPRESSION; ALSO COMPRESSION AND PULL COMBINED

KNIGHT (**14**) tied a young tree in such a manner that it was swayed only in the plane of the prevailing wind. After the lapse of one growing season, the diameter in this plane was found to be greater than the diameter at right angles to the same.

HIBBARD (**11**) found that compression caused a small increase of mechanical tissue in the stems of Fuchsia, Vinca, and Helianthus, while Coleus gave no response.

3. ROOTS: RESPONSE TO TENSION

HIBBARD (**11**) says: "Pull in the direction of the longitudinal axis of the plant called forth a small increase of mechanical tissue in the main and lateral roots of *Helianthus annuus* and *Ricinus communis*."

4. TENDRILS AND PETIOLES

DARWIN (**4**; GRAY **8,** p. 176) says that in the grape-vine, Virginia creeper, etc., attached tendrils thicken and harden, gaining wonderfully in strength and durability, while those which remain unattached soon shrink up or wither and fall off.

MÜLLER (**18**) found that contact produced earlier and greater lignification of the sclerenchyma, even in the free portion of the ten-

drils of the Cucurbitaceae; but he did not consider tension as a probable factor along with contact.

WORGITZKY (**30**) reports that tendrils of *Passiflora quadrangularis* which had grasped a support broke at 600^{gm}, while the controls broke at 350^{gm}. With tendrils of Cucurbitaceae, the uncoiling resistance was thirteen times as great in those which had grasped a support. There was also a marked anatomical change; viz., a lignification of the pith parenchyma, which he assigned to no cause.

VON DERSCHAU (**28**) found that a gradually increasing pull on certain twining petioles raised their breaking strength and caused an increased development of mechanical tissue. He found a numerical increase in almost all kinds of cells. The petioles of *Solanum jasminoides* showed the least response.

NEWCOMBE (**19,** p. 446) says, in speaking of the reaction of tendrils to contact: "The first strengthening tissue is here laid down as a response to contact; its increase is the regulatory response of the plant to the strain that it feels."

MACDOUGAL (**17,** pp. 377, 378) believes that contact stimuli are not transmitted beyond 2 or 3^{mm}. Since the thickening of the tendril always takes place after contact, the natural conclusion would be that this thickening of the tendril is due to the traction exerted by the weight of the stem supported by it.

PEIRCE also (**20,** p. 241) believes that the strengthening of the free basal portion is not due to contact.

FITTING (**6,** p. 476) has demonstrated that contact stimuli are transmitted to the basal portion of the tendril, and that therefore the effect of contact stimuli on the basal portion of tendrils cannot be excluded.

5. CORRELATION

The factor of correlation has been investigated by numerous workers. Since self-regulatory development may imply a certain degree of correlation, it seems desirable that at least a brief review of the work on correlation should be given.

LABORIE (**16**) found decided differences in the anatomical structure of the floral axis compared with the rest of the plant: (1) an increase of bast, (2) a decided modification of the main bundles, and (3) a decrease of pith.

KLEIN (13) found the bundles more centrally located in the fruit stalk than in the petiole. He attributed this arrangement to the necessity for a greater mechanical strength, as well as for a more abundant supply of building material.

DENNERT (5) compared the anatomical structure of the fruit stalk before and after the ripening of the fruit. He found an increase in the development of mechanical tissue. There was an increased amount of xylem and a greater thickness of the walls of the wood fibers.

REICHE (24) investigated the transformation from flower to fruit stalk in many additional plants. His conclusions agree with those of the earlier investigators.

PIETERS (23) showed that one-year-old fruit-bearing shoots of the apple and the pear had a smaller xylem cylinder in proportion to their diameters than the vegetative shoots of same age. They were well supplied with supplementary mechanical tissue, however, which was distributed at those points where it was most needed. In the case of the peach and the plum, the woody cylinder of the fruit-bearing shoot was larger than in the vegetative shoot. There was an abundance of well-lignified sclerenchyma and hard bast in the fruit-bearing shoots of the apple and pear, while in the vegetative shoots these tissues appeared only sparingly, if at all.

GOEBEL (7, p. 206) writes: "Careful research demonstrates the existence of reciprocity between parts of the plant body. . . . . The size and construction of one organ are frequently determined by those of another."

BOODLE (3) states that the walls of the sieve tubes and companion cells in *Helianthus annuus* become lignified as a result of strain. He also found a slight lignification of the parenchymatous parts of the pericycle and medullary rays, thus uniting the primary sclerenchyma strands into a more definite mechanical system attached to the strong xylem by the medullary rays. He says: "This must give greater rigidity, which no doubt is required by the heavy fruiting capitula borne by the plants."

KELLER (12) reached conclusions directly opposed to those reached by BOODLE. He showed that a strong or light pull in the direction of the longitudinal axis of orthotropic flower stalks exerted

no influence on the development of mechanical tissue. The displacement of orthotropic flower shoots to a plagiotropic position, he claimed, caused a certain anatomical change. This he attributed to the change in position and not to tension. His conclusion does not agree with those of other investigators, viz., that mechanical development of the fruit stalk goes hand in hand with the development of the fruit.

Methods

In both greenhouse and field culture a much larger number of plants were grown than were used in each experiment. At the beginning of each experiment the strongest plants and those most nearly equal in size were selected, and the others were removed. This was done to reduce the chances, as far as possible, of individual variation.

In the greenhouse cultures, the pots with the plants were placed in a row. The plants were then measured and every other one was taken as a control. At the close of the experiment, an average was taken of all the data on the control, and also the experimental plants in each series. This was the method used by SCHOLTZ. By preliminary experiments, the writer was convinced that it was by far the most satisfactory, on account of individual variations in plants which make it very difficult to select any one plant that will be a fair control throughout the experiment for any one experimental plant.

The methods of BALL and HIBBARD for applying tension by pull in the direction of the longitudinal axis of the stem were followed. The writer was soon convinced, however, that for plants as large as those used in his experiments, the leather noose used by BALL as a tension fastening was not needed. Heavy cotton flannel and ordinary cotton cord, therefore, were used for this purpose in all these experiments. A strip of heavy cotton flannel 2.5^{cm} wide was wrapped at least twice around the stem. Over this, two cords were passed from opposite sides and back again. Each cord, thus passing twice around the stem, was drawn tight enough to keep the fastening from slipping. The ends of each cord were then tied to a second. These were united into one strand some distance above the fastening. This double cord was passed over a light running pulley, supported directly above the plant, and a weight was attached to its free end. A thin

strip of wood or bamboo about 10^{cm} long was placed crosswise between the cords coming up on opposite sides of the stem, to keep them from injuring the young leaves and growing tip of the stem.

In the field cultures, exactly the same methods were followed, except that the seeds were planted in rows. The inferior seedlings were removed, and only the stronger ones were left for experimental use. In most cases the heavy clay loam was sufficiently firm to keep the plants from being pulled up by the tension. In the few cases when the soil was not firm enough, heavy flat weights were used to hold it in place.

METHODS IN FINAL DETERMINATIONS

Method 1.—In all but two experiments the stems were tested by direct pull along their longitudinal axes to determine whether they had responded in a self-regulatory manner to traction by increasing their breaking strength.

Preliminary experiments convinced the writer that the method of obtaining the breaking strength used by BALL was not satisfactory, since too many of the experimental plants broke at or just below the tension fastening. Larger plants therefore were used. These were broken at some distance below the tension fastening, thus eliminating the unquestionably weakening effect coming from the pressure of a tension fastening on the young and tender stem.

For this purpose, a strong wooden frame was constructed, about 120^{cm} long and 50^{cm} high. Through the middle of the frame ran a shelf parallel with top and bottom. On this shelf rested the plant, blocks, and spring balance used in determining the tensile strength. To grasp the ends of the stem, 4 blocks of wood were selected, each being 5^{cm} thick, 10^{cm} wide, and 20^{cm} long. These blocks were laid together in pairs, one block above its mate, and grooves considerably larger than the stems were cut in the contact faces of each pair to receive the plant stem.

During the preliminary experiments, the stems, except in the portion to be tested, were wrapped in moist cotton and then imbedded in a damp mixture of sand and Portland cement. This mixture was placed in the groove of the lower half of the pair of blocks. The stem was then placed in position and more concrete was piled on top.

This was pressed down and around the stem by tightening the bolts which were used to draw the two blocks together. This method was satisfactory. It was soon found, however, that the stem could be held sufficiently firm by substituting moist cotton for the mixture of sand and Portland cement. Moist cotton, therefore, was used in all the experiments as packing to fasten the stem in the blocks.

When the stems were fastened in the blocks, one pair of blocks was hooked to the spring balance, and the other pair was secured to one end of the wooden frame. The spring balance was hooked in turn to an iron rod passing through the opposite upright of the wooden frame. On the end of this rod, protruding beyond the frame, was a large tail-nut. By turning this tail-nut with the hand, tension was brought on the spring balance, and through the balance on the plant stem. The breaking strength was read from the face of the spring balance.

After the stems were broken they were preserved either in 50 per cent. alcohol, or short sections were cut from just below the breaking point in the stems, and these were killed and fixed in 1 per cent. chrom-acetic acid, thoroughly washed, dehydrated in alcohol, and kept for future study.

The following methods were used in making microscopic examinations and measurements of the xylem and hard bast in the stems.

Method 2.—Measurements of the thickness of cell walls in stained free-hand sections, and also in some cases of microtome sections, were made by using an ocular micrometer. The results recorded are in each case an average of eight measurements taken in as many distinct areas of the cross-section.

Method 3.—The total xylem and hard bast areas were measured in some of the experiments by projecting a cross-section of the stem upon a bristol-board screen, and measuring the areas with a polar planimeter.

Method 4.—Camera lucida drawings of the total xylem and hard bast areas and also of the hard bast elements were made upon standardized bristol-board. The drawings were very carefuly cut out with a sharp and pointed scalpel, and then weighed on a chemical balance. The number of hard bast elements were also counted by fastening the drawings of the hard bast elements over sheets of yellow

paper and checking off each element by marking through the lumen on the yellow paper underneath.

Method 5.—The hard bast elements were drawn by means of a camera lucida and then counted to determine whether an increase had resulted in response to the pull.

Method 6.—Radial and tangential microtome sections of acid-fixed material were made to determine whether any fibrous parenchyma existed directly around the hard bast areas. This method was used only on control stems of *Helianthus annuus*. It was the purpose to determine whether the elements which produced the increased number of bast fibers were present before the tension was applied and therefore only developed, or whether they were formed *de novo* in response to tension.

The plants investigated were *Helianthus annuus*, *Phaseolus vulgaris*, *Ricinus communis*, *Sinapis alba*, *Vicia Faba*, *Lupinus albus*, *Rubus occidentalis*, and *Vinca major*.

Experiments

Experiment 1. Helianthus annuus, greenhouse culture.—Ten vigorous plants were selected from a number of seedlings grown in pots 20^{cm} in diameter. The experiment began November 21, 1906, when they were mostly 10 to 15^{cm} in height, and ended 29 days later. The tension fastening was placed on the experimental plants just below the second pair of leaves. The controls were not supported. The pull applied to the experimental plants was gradually increased from 25^{gm} at the start to 300^{gm} on December 14, 1906. The slow growth of these plants may be due to the fact that during the entire experimental period there were only a few sunny days, and also to the shortness of the day at this season of the year.

Table I gives all the data from this experiment, except the following: By method 6, fibrous parenchyma was found in the control stems as a transitional form between the thick-walled hard bast fibers and the short-celled parenchymatous tissue. These longitudinal sections were taken from just above the cotyledons. The increased number of bast fibers in the experimental plants, therefore, may be accounted for as the result of the response of these fibrous cells to tension, which thus develop into hard bast fibers.

The actual area of the cell walls of the hard bast elements and their number was found by method 4, and the total xylem area by method 3.

TABLE I.—*Helianthus annuus*

No. of plant	November 21		November 28		December 14		December 20		Breaking strength in grams	Cell wall area of hard bast elements in grams	No. of hard bast fibers	Xylem area in sq. cm. × 10
	Height in cm.	Diam. in mm.	Height in cm.	Diam. in mm.	Height in cm.	Diam. in mm.	Height in cm.	Diam. in mm.				
Exp.												
1.....	19.	4.	29.	4.	50.4	4.	59.2	4.2	7711	6.837	691	8.8
2.....	13.1	3.2	19.	3.2	34.	4.1	41.6	4.2	5443	7.014	811	6.6
3.....	10.6	3.5	19.	3.5	37.8	3.6	44.7	3.5	5216	6.430	840	4.1
4.....	11.8	3.1	20.7	3.2	35.3	3.6	44.1	3.9	4626	5.488	682	8.8
5.....	9.	2.	20.2	2.	36.5	3.	44.1	3.2	5443	5.666	581	5.2
Total	63.5	15.8	107.9	15.9	194.0	18.3	233.7	19.0	28749	31.435	3605	33.5
Av....	12.7	3.16	21.58	3.18	38.8	3.66	46.74	3.8	5749.8	6.287	721	6.7
Control												
1.....	15.2	3.	22.7	3.	42.8	4.1	52.9	4.3	5352	5.145	597	6.6
2.....	11.3	3.5	17.7	3.5	34.	3.8	42.8	4.	3039	6.420	706	4.3
3.....	11.8	2.5	19.	3.	34.	3.4	41.6	3.5	3719	5.372	657	5.9
4.....	11.3	3.2	18.3	3.2	34.	3.2	39.	3.5	2494	5.498	632	3.7
5.....	9.	2.2	12.1	2.2	23.3	3.2	30.	3.4	3629	4.679	656	3.3
Total	58.6	14.4	89.7	14.9	168.1	17.7	206.3	18.5	18233	27.114	3248	23.8
Av....	11.72	2.88	17.94	2.98	33.62	3.54	41.26	3.7	3647	5.423	641	4.76

The percentages of increase in the experimental plants over the controls are the following: breaking strength 57.6 per cent.; cross-section of walls of hard bast 16 per cent.; number of hard bast elements 12.5 per cent.; and xylem area 40 per cent.

TABLE II.—*Helianthus annuus*

No. of plant	Height in cm.							Height to 2d pair of leaves	Diam. just above 1st pair of leaves in mm.		Distance of break below 2d pair of leaves	Breaking strength in kg
Exp.	June 21	June 22	June 24	June 26	June 28	June 30	July 1	July 1	June 21	July 1	July 1	July 1
Av.....	7.5	8.6	12.6	16.3	19.4	23.8	26.6	10.4	4.1	7.26	3.25	16.535
Control												
Av.....	7.03	8.2	11.3	14.3	17.3	21.5	24.3	9.2	4.1	7.43	3.72	13.799

Experiment 2. Helianthus annuus, field culture.—After the inferior plants had been removed, those used in this experiment stood 15 to 20^{cm} apart in the row. Every other plant was used as a control. The tension fastening was placed just below the second pair of leaves on the experimental plants. The control plants were left free. The experiment began June 21, 1907, and ended ten days later. A tension of 250^{gm} was applied to the experimental plants at the beginning of the experiment. This was increased to 750^{gm} the following day and to 1900^{gm} on June 25.

Table II gives an average of the measurements and the breaking strength of these plants. The average increase in breaking strength for the experimental plants over the controls was 2736^{gm} or 19.6 per cent.

The height measurements in this experiment confirm the work of SCHOLTZ and HEGLER (**'93**), showing a lessening of growth during the first 24 hours, followed by an increased growth for the next four days.

TABLE III.—*Helianthus annuus*

No. of plant	Height in cm.		Height to 4th pair of leaves		Diam. just above 1st pair of leaves in mm.		Diam. just above 2d pair of leaves in mm.		Distance of break above ground in cm.	Breaking strength in kg.
Exp.	July 18	July 30	July 18	July 30	July 18	July 30	July 18	July 30	July 30	July 30
Av.....	36.07	89.6	32.33	44.6	9.4	15.95	7.58	13.39	12.3	69.99
Control										
Av.....	38.01	90.5	34.6	45.08	9.64	16.03	7.69	14.37	13.9	66.12

Experiment 3. Helianthus annuus, field culture.—Much older and larger plants than those used in experiment 2 were put under tension July 18, 1908. This was gradually increased from 200^{gm} at the start to 1950^{gm} on July 27. The experiment ended July 30. It was the purpose in this experiment to determine whether the same tension would have the same influence on larger and older stems as on younger and less woody ones. The average increase in breaking strength as shown in table III in response to a tension of practically 1900^{gm} was 3.87^{kg}, while in experiment 2 the increase for a period of 10 days was 2.74^{kg}. The increase as a percentage of the average

breaking strength of the controls in this experiment, 5.8 per cent., was much less than in experiment 2, since the breaking strength was so much greater.

The tension fastenings were made just below the fourth pair of leaves. Similar fastenings were made on the control plants, and from these the plants were fastened to upright supports to keep them from being swayed by the wind.

Experiment 4. Sinapis alba, field culture.—These plants grew approximately 15^{cm} apart in the row. Every other plant was used as a control. The tension fastening was placed just below the seventh node above ground. Similar fastenings were placed on the control plants. From these fastenings, the plants were attached to strong cords, stretched one on each side of the row and parallel to it. A weight of 250^{gm} was attached to the experimental plants on July 16, 1907, and gradually increased to 1150^{gm} on July 24. The experiment ended two days later. The details of this experiment are given in table IV. The average increase of the breaking strength of the experimental over the control plants was 4.8^{kg} or 32 per cent.

The anatomical structure of these stems was determined by methods 2, 3, 4, and 5. Free-hand sections were made of all the stems about 5^{mm} below the point where they broke. These sections were stained in anilin safranin and mounted in glycerin. All the slides were then taken by a second party who labeled them with a secret label. The measurements given in table IV were then made, after which the second party gave me the key to the labels. The measurements of the experimental plants were now separated from those of the controls. An average of each set shows a decrease of 10 per cent. in the total xylem area and an increase in the thickness of the xylem cell walls of 5 per cent. The increase in hard bast in 5 stems which were selected wholly by chance was 52 per cent. The increase of hard bast elements in the same stems was 38 per cent.

The total xylem area for the five stems in which the hard bast determinations were made was also measured by method 4, with results that agreed within less than one-half of 1 per cent. with the measurements made by method 3.

The hard bast in the controls was lignified in all but a few stems, where it was only partly lignified. Stem 18 showed the least lignifi-

TABLE IV.—*Sinapis alba*

No. of plant	Height in cm.		Height to fastening in cm.		Diam. just above ground in mm.		Distance of break above ground in cm.	Breaking strength in kg.	Cell wall area of hard bast elements in grams	No. of hard bast elements	Xylem area in sq. cm. × 10	Average thickness of xylem cell walls in microns
Exp.	July 16	July 26	July 16	July 26	July 16	July 26	July 26	July 26	July 26	July 26	July 26	July 26
1	18.3	60.	15.	23.	6.85	9.25	16	28.09	.....	.....	15.88	2.812
2	25.	55.5	19.	21.5	5.1	7.3	13	16.76	.....	.....	6.99	2.625
3	22.	56.0	17.2	19.	5.	6.07	8	21.29	.....	.....	7.62	4.312
4	14.8	48.5	12.	16.	4.65	7.5	7	24.46	.....	.....	12.70	3.437
5	19.	53.5	14.	18.	5.1	7.15	12	11.79	.....	.....	9.84	2.500
6	23.5	60.	18.5	23.	5.95	8.8	13	21.74	.....	.....	8.67	2.562
7	20.5	57.	15.5	21.	7.2	9.35	13	12.23	.....	.....	8.89	2.812
8	24.	70.	20.	22.	7.4	9.9	16	16.76	11.395	656	10.70	2.562
9	26.4	72.	21.8	30.5	7.45	9.33	20	24.92	11.801	861	11.11	3.124
10	24.	68.	21.	31.	6.05	7.32	21	28.09	7.061	561	9.53	3.625
11	27.	69.	22.7	29.	5.85	8.3	16	22.20	.....	.....	10.70	3.375
12	27.5	67.	20.5	26.	6.55	8.65	14	32.62	.....	.....	8.89	4.625
13	27.	72.	21.	29.	5.75	7.65	12	16.76	.....	.....	9.84	3.687
14	24.7	62.	20.8	26.	6.45	8.1	15	17.21	.....	.....	12.70	3.375
15	27.5	62.	19.8	21.	5.4	5.77	12	17.21	5.938	578	3.18	3.037
16	26.3	68.	23.	26.	47.	6.75	7	25.82	4.598	526	7.94	2.875
17	25.8	59.	21.5	26.	4.6	5.2	15	11.33	.....	.....	5.40	2.625
18	28.3	67.	22.	25.	5.15	6.5	8	18.57	.....	.....	8.26	3.000
19	17.	46.	14.	19.	4.1	7.1	7	7.70	.....	.....	9.53	2.750
Total	503.0	1172.5	359.3	452.0	109.3	145.99	245.	375.55	40.791	3182	178.37	59.720
Average	23.6	61.7	18.9	23.8	5.75	7.68	12.9	19.77	8.158	636	9.39	3.143

TABLE IV.—*Sinapis alba (continued)*

No. of plant	Height in cm.		Height to fastening in cm.		Diam. just above ground in mm.		Distance of break from ground in cm.	Breaking strength in kg.	Cell wall area of hard bast elements in grams	No. of hard bast elements	Xylem area in sq. cm. ×10	Average thickness of xylem cell walls in microns
Control	July 16	July 26	July 16	July 26	July 16	July 26	July 26	July 26	July 26	July 26	July 26	July 26
1	23.4	55.5	20.	24.	5.10	7.25	15.	15.40	.....	.....	7.30	3.187
2	26.3	63.	20.5	24.5	6.10	8.60	7.	15.41	.....	.....	11.43	3.062
3	17.2	50.	14.6	17.5	4.15	5.75	13.	9.97	.....	.....	3.81	2.562
4	20.3	63.	15.4	19.5	5.60	7.70	12.	19.94	.....	.....	9.53	2.937
5	18.8	58.	14.5	19.5	5.35	8.22	15.	19.94	7.075	542	12.70	3.062
6	26.	60.	22.5	34.	8.00	10.62	19.	10.41	.....	.....	19.05	3.000
7	22.7	64.	17.5	21.5	7.20	9.35	10.	11.79	4.714	365	16.51	2.562
8	24.4	63.	19.5	22.	6.20	8.73	7.	11.33	.....	.....	7.62	3.000
9	21.7	66.	16.3	22.	7.00	8.63	5.	15.86	.....	.....	10.80	3.062
10	26.3	66.	22.3	33.5	7.15	10.30	19.	21.74	.....	.....	19.69	2.437
11	21.9	58.	18.0	20.	5.15	6.10	8.	9.51	.....	.....	6.35	3.437
12	28.5	70.	28.3	34.5	6.30	7.00	17.	19.03	5.709	510	8.25	2.875
13	28.7	62	21.4	24.	5.60	6.25	7.	13.13	.....	.....	6.35	4.062
14	25.8	66.	21.5	28.	5.80	7.72	13.	16.76	.....	.....	11.43	3.375
15	27.7	79.	21.5	24.	5.00	6.55	8.	12.68	5.040	491	6.67	3.125
16	24.5	57.	20.5	24.	6.10	7.80	7.	17.21	.....	.....	11.43	2.500
17	31.5	73.	25.0	27.	5.20	6.00	12.	25.86	.....	.....	6.03	3.062
18	26.3	51.	21.8	23.	4.40	7.50	13.	5.54	4.159	395	8.89	2.500
19	26.5	61.	22.5	24.	5.40	7.45	8.	14.50	.....	.....	13.07	3.500
20	19.5	53.	16.3	20.	4.80	9.10	10.	13.59	.....	.....	13.27	2.561
Total	488.0	1237.5	396.9	486.5	115.60	156.92	225.	298.60	26.697	2305	205.19	59.868
Average	24.4	61.87	19.84	24.32	5.78	7.85	11.25	14.93	5.339	461	10.51	2.993

cation among the controls. Stems 5, 7, 12, and 15 were all well lignified. The hard bast in approximately 10 per cent. of the experimental stems was completely lignified. In the five stems for which the hard bast determinations were made, only a few elements were lignified in 8 and 9, while in 16 lignification of the hard bast was almost complete.

Experiment 5. Phaseolus vulgaris, greenhouse culture.—The plants for this experiment were grown and selected in a like manner to those for experiment 1. The tension fastening was placed just below the second pair of leaves. The experiment began October 21 and ended November 8, 1907. The weight attached to the experimental plants, 400^{gm} at the beginning, was increased to 950^{gm} after five days, and to 1150^{gm} November 5. The controls were left unsupported.

TABLE V.—*Phaseolus vulgaris*

No. of plant	Height in cm.		Height to 2d pair of leaves in cm.		Diam. just above ground in mm.		Diam. just above cotyledons in mm.		Place where stem broke	Breaking strength in kg.	Bast area in sq. cm. ×125	Xylem area in sq. cm. ×20
	Oct. 21	Nov. 8	Oct. 21	Nov. 8	Oct. 21	Nov. 8	Oct. 21	Nov. 8	Nov. 8	Nov. 8	Nov. 8	Nov. 8
Exp. Av.....	13.5	25.	12.1	14.1	4.34	4.82	3.1	3.3	.....	6.795	88.08	6.59
Control Av.....	13.3	20.8	12.0	12.8	4.16	4.53	2.98	3.34	.....	5.098	76.57	5.42

The average measurements and results of this experiment are given in table V. The breaking strength shows an increase in the experimental plants over the controls of 1.7^{kg}, or 33 per cent. Sections taken just below the breaks showed an increase of 15 per cent. in the area of hard bast, and 22 per cent. in the area of the xylem. These measurements were made by method 3. The hard bast and xylem were equally lignified in experimental and control plants.

Experiment 6. Phaseolus vulgaris, greenhouse culture.—These plants, when the experiment was started, were ten days older than those used in experiment 5. The experiment began December 7, 1907, and ended January 7, 1908, at which date most of the blossoms had already fallen from the plants and the leaves were beginning to lose their chlorophyll. A weight of 250^{gm} attached to the experi-

mental plants at the beginning of the experiment was gradually increased to 900^{gm} on December 20. It was the purpose of this experiment to determine, by prolonging the experimental period and growing the plants to maturity, whether the response in the earlier and actively growing period was continued to the end of the vegetative period. For this reason no additional weights were added after December 20. Both experimental and control plants broke with almost the same weight. The experimental plants were much more brittle and in most cases broke with a smooth, clear break, while the controls showed more of the elasticity and toughness which was characteristic of the stems in experiment 5.

Experiment 7. Phaseolus vulgaris, greenhouse culture.—After finding that response to tension, if any had occurred in experiment 6, was vitiated by permitting the plants to grow to maturity, experiment 5 was repeated, beginning March 5 and ending fourteen days later. The weights used, 200^{gm} at the beginning, were increased to 450^{gm} March 9 and to 700^{gm} four days later. The experimental plants showed an average increase in breaking strength of 3.32^{kg}, or 42.5 per cent. The number of hard bast elements was determined by method 5. The experimental plants showed an average increase of 167, or 14.9 per cent. The results of this experiment, therefore, agree with those of experiment 5. The tension fastening in this and also in experiment 6 was made at the same place as in experiment 5.

Experiment 8. Vinca major, greenhouse culture.—In selecting plants for this experiment those were taken which had young and actively growing shoots, uniform in general appearance and length. No measurements were taken at the beginning of the experiment. December 1, 1906. The experimental shoots were put under a tension of 50^{gm} at the beginning, which was gradually increased to 300^{gm} on December 21. The control plants were fastened to an upright support, but were not under tension.

The breaking strength was tested in the internode just below the fastening which was in an active growing condition in each plant when the experiment was started. The average shows an increase in the experimental plants of 649^{gm}, or 15.2 per cent.

Experiment 9. Vinca major, greenhouse culture.—It was the purpose of this experiment to determine whether internodes which

were no longer elongating would respond to tension by increasing their breaking strength. For this reason, shoots older and larger than those in experiment 8 were used. The controls were supported as in experiment 8. A weight of 200^{gm} attached to the experimental shoots on November 15, 1907, was increased to 400^{gm} on December 14. The experiment ended January 8, 1908.

The third instead of the last internode below the tension fastening was tested for its breaking strength. No elongation and in many cases no growth in diameter took place in this internode. An average of the breaking strengths shows no response to tension for an internode which is no longer actively growing.

TABLE VI.—*Vinca major*

RECORD A

No. of plant	Height in cm.		Height to node just above fastening in cm.		Diam. just below fastening in cm.		No. of node just above fastening	Distance of breaking point below fastening in cm.	Breaking strength in kg.	Thickness of xylem cell walls in microns	Weight of xylem area in grams	Thickness of hard bast fibers in microns	No. of hard bast fibers
Exp.	Jan. 9	Jan. 25	Jan. 9	Jan. 25	Jan. 9	Jan. 25			Jan. 25				
Av....	29.	41.4	25.6	27.3	2.11	2.22	.....	8.25	4.53	.....	.....	.....	.....
Control													
Av....	32.	44.	28.4	29.6	2.09	2.20	.....	8.75	4.24	.....	.....	.....	.....

RECORD B

Exp.		Mar. 2		Mar. 2		Mar. 2			Mar. 2	Mar. 2	Mar. 2	Mar. 2	Mar. 2
Av...	29.2	95.2	27.	29.1	2.05	2.38	.....	15	8.22	5.77	.2777	7.42	1276
Control													
Av...	28.6	62	26.	28.5	2.1	2.32	.....	12.1	6.97	5.09	.2140	6.56	1297

Experiment 10. Vinca major, greenhouse culture.—This experiment was in large measure a repetition of experiment 8. Tension fastenings were made on all the shoots. Just enough weight was attached to the control shoots to hold them erect. The experiment began January 9, 1908, with a weight of 250^{gm} attached to the experimental plants. This was increased to 450^{gm} eight days later, and to 900^{gm} January 25. The internode to which the tension fastening was attached was in each case actively elongating at the beginning of the

experiment. One-half of the shoots were removed and tested January 25, i. e., after 16 days and subjection to a maximum tension of 450^{gm}. Table VI, record A, gives an average of the results for this part of the experiment. The average breaking strength is slightly increased in the experimental shoots.

The remaining shoots were tested March 2, 1908. An average of breaking strengths as given in table VI, record B, shows an increase in favor of the experimental shoots of 1.25^{kg} or 18 per cent. A study of the anatomical structure of these stems was made by methods 2, 4, and 5. These determinations show an increase of 30 per cent. in the entire xylem area, an increase in xylem wall thickness of 13 per cent., and the same increase in the thickness of the walls of the hard bast fibers. A count of the hard bast fibers showed no increase in the number of these elements.

Experiment 11. Ricinus communis, field culture.—The method in this experiment was the same as in experiment 2. The tension fastening was placed just below the second pair of leaves. The control plants were not supported. The experiment began July 20 and ended August 1. A tension of 300^{gm} at the beginning was gradually raised to 2350^{gm} two days before the experiment ended.

TABLE VII.—*Ricinus communis*

No. of plant	Height in cm.		Height to 2d pair of leaves in cm.		Diam. at base in mm.		Diam. just above 1st pair of leaves in mm.		Breaking strength in kg.	Distance of break from ground in cm.	Xylem area in sq. cm. ×10	Av. xylem cell wall thickness in microns
Exp.	July 20	Aug. 1	July 20	Aug. 1	July 20	Aug. 1	July 20	Aug. 1	Aug. 1	Aug. 1	Aug. 1	Aug. 1
Av.....	24.4	44.83	16.25	17.77	8.18	12.18	10.66	13.01	43.86	10.24	27.07	3.038
Control Av.....	22.8	43.8	16.4	19.6	8.44	11.41	9.95	12.56	41.22	10.4	25.99	2.929

The average results, given in table VII, show 43.86^{kg} as the average breaking strength for the experimental plants and 41.22^{kg} for the controls, or 6.4 per cent. The increase is slightly greater than the final weight which was attached to the experimental plants. The average xylem wall thickness and total xylem area, as determined by methods 2 and 3, show an increase respectively of 0.1μ and 4 per cent.

The bast fibers were not well defined; many of them only very slightly thickened. It was therefore impossible to make an accurate count of their number.

Experiment 12. *Rubus occidentalis*, field culture.—On June 25, 1907, ten young stems were selected which in general appearance were equally vigorous. Tension fastenings were placed on five of these approximately 35^{cm} from the ground. The controls were left free. A weight of 2.25^{kg} at the beginning was increased to 4.5^{kg} on July 6, and to 9^{kg} 24 days later. The experiment ended September 30.

The decrease in height in the experimental plants was very marked. Growth in diameter was also less. The total bast and xylem areas were determined by method 3, giving an average increase in bast of 13.6 per cent. over the controls, but a decrease in xylem of 30 per cent. This decrease may be partly attributed to the reduced growth in diameter of the experimental plants.

Experiment 13. *Vicia Faba*, greenhouse culture.—The experimental plants were put under tension on October 24, 1907, the fastening being placed just below the fifth node. Since *Vicia Faba* grows very rapidly, it was thought possible that it might respond to a stronger tension than that applied to stems of other species of like cross-section and age. A weight of 450^{gm}, therefore, was applied at the beginning, increased to 900^{gm} two days later, and to 1300^{gm} on November 6. Since illumination is greatly reduced at this time of the year and growth is comparatively slow, this large weight caused the stem to elongate more rapidly than the control. This was accompanied by diminished growth in diameter. The control plants were left free, and since *Vicia Faba* retains its orthotropic position with great difficulty, these grew larger in diameter and less in height. When the experiment ended on November 9, the breaking strength of the experimental plants was less than the breaking strength of the controls.

Experiment 14. *Vicia Faba*, greenhouse culture.—The results of experiment 13 gave cause for this experiment, in which an effort was made to eliminate or at least minimize the effect of those factors which may have obscured the response to tension in the last experiment, if such a response did occur. For this reason, the experiment was carried on during the season of the year when illumination was much stronger and hence the chances for growth much better, all other

factors affecting growth remaining unchanged. Smaller weights also were used on the experimental plants, and the control plants were supported by small weights, barely sufficient to counterbalance the weight of plant, attached as the larger weights were to the experimental plants, the cords running over pulleys. The experimental and control plants were now growing under exactly the same conditions, except that the former were subject to tension of 200^{gm} at the beginning, increased to 450^{gm} on March 19, and to 700^{gm} on March 23, and finally to 950^{gm} on March 30. The experiment ended April 6.

TABLE VIII.—*Vicia Faba*

No. of plant	Height in cm.		Height to 4th node in cm.		Diam. just above 1st leaf in mm.		Diam. just below 4th node in mm.		Distance of breaking point above ground in cm.	Breaking strength in kg.	No. of hard bast fibers	Xylem area in sq. cm. ×18
Exp.	Mar. 14	Apr. 6	Mar. 14	Apr. 6	Mar. 14	Apr. 6	Mar. 14	Apr. 6	Apr. 6	Apr. 6	Apr. 6	Apr. 6
Av.....	21.3	69.8	15.9	20.3	5.6	8.49	4.82	6.33	8.8	24.91	1630.3	22.75
Control Av.....	22.4	66.	15.3	18:0	6.01	8.40	4.80	6.39	9.3	20.59	1419.4	21.00

The results are given in table VIII. The breaking strength shows an increase of 4.35^{kg} or 21 per cent. for the experimental plants over the controls. The number of hard bast fibers, determined by method 5, and the total xylem area by method 3, show an increase in the former of 14.8 per cent., and in the latter of 8.3 per cent.

TABLE IX.—*Lupinus albus*

No. of plant	Height in cm.		Height to cotyledons in cm.		Diam. half-way between ground and cotyledons in mm.		No. of hard bast fibers	Xylem area in grams
Exp.	Mar. 2	Mar. 30	Mar. 2	Mar. 30	Mar. 2	Mar. 30	Mar. 30	Mar. 30
Av.....	4.74	9.02	3.54	5.06	3.71	2.91	839.4	.9127
Control Av.....	4.7	6.95	3.55	3.97	3.70	2.97	755	.800

Experiment 15. Lupinus albus, greenhouse culture.—This experiment began March 2 and ended March 30, the experimental plants

being subjected to a tension of 250^{gm} at the beginning, increased to 500^{gm} seven days later, and to 750^{gm} on March 19. On account of the shortness of the stems, which made it impossible to fasten them into the apparatus firmly enough to hold a sufficient weight, the attempt to determine the breaking strength of these stems was unsatisfactory. The reduced diameter, in that part of the stem below the cotyledons as shown in table IX, was due to a collapse of the tissue surrounding the stele. The total xylem area and number of hard bast elements, determined by methods 4 and 5, show an average increase of the former to be 14 per cent. and of the latter 11 per cent.

Summary

An average of the different determinations made in the foregoing experiments is given in table X. In all except experiment 9 with *Vinca major* and experiment 13 with *Vicia Faba*, the average breaking strength in the 246 tests shows an increase in the experimental plants over the controls. This must be attributed to a response to traction along the longitudinal axis of the stem. The negative results in experiment 9 show that the older part of the stem, where active growth has ceased, does not respond by increasing its breaking strength. The lack of response in experiment 13 with *Vicia Faba* can be attributed to factors which vitiated the influence of tension. These conditions are fully explained in experiments 13 and 14. Experiments 9 and 13, therefore, may be left out of consideration. The average area of the cell wall of the hard bast elements shows an increase in the experimental plants over the controls for *Helianthus annuus* and *Sinapis alba*. This determination was not made for the other species investigated. The average number of hard bast elements shows an increase in the experimental plants over the controls in all the species for which this determination was made, except *Vinca major*. *Phaseolus vulgaris* and *Rubus occidentalis* also show an increase of total bast area in the experimental plants over the controls. The average thickness of the hard bast fibers in *Vinca major* was found to be greater for the experimental stems.

In the total xylem area determinations, the only decrease was in *Sinapis alba* and *Rubus occidentalis*, in both of which, however, there

was an increase in hard bast. The other species all responded to tension by increasing their xylem area. The average thickness of

TABLE X.—SUMMARY

No. of experiment	Name of plant	Average breaking strength in kg.	Average cell wall area of hard bast fibers	Average no. of hard bast fibers	Average total hard bast area	Average thickness of hard bast cell walls	Average total xylem area	Average thickness of xylem cell walls	No. of stems used
Exp. 1...	Helianthus annuus	5.750	6.287	721	.....	.....	6.70	....	5
Cont. 1..	Helianthus annuus	3.647	5.423	641	.....	.....	4.76	.....	5
Exp. 2...	Helianthus annuus	16.535	.....	.....	.....	.....	.....	.....	12
Cont. 2..	Helianthus annuus	13.799	.....	.....	.....	.....	.....	.....	13
Exp. 3...	Helianthus annuus	69.990	.....	.....	.....	.....	.....	.....	12
Cont. 3..	Helianthus annuus	66.120	.....	.....	.....	.....	.....	.....	11
Exp. 4...	Sinapis alba	19.770	8.158	636	.....	.....	9.39	3.143	19
Cont. 4..	Sinapis alba	14.930	5.339	461	.....	.....	10.51	2.993	20
Exp. 5...	Phaseolus vulgaris	6.795	.....	.....	88.08	.....	6.59	.....	4
Cont. 5..	Phaseolus vulgaris	5.098	.....	.....	76.57	.....	5.42	.....	4
Exp. 6...	Phaseolus vulgaris	5.850	.....	.....	.....	.....	.....	.....	11
Cont. 6..	Phaseolus vulgaris	5.570	.....	.....	.....	.....	.....	.....	13
Exp. 7...	Phaseolus vulgaris	10.135	.....	1288	.....	.....	.....	.....	4
Cont. 7..	Phaseolus vulgaris	7.815	.....	1121	.....	.....	.....	.....	4
Exp. 8...	Vinca major	5.903	.....	.....	.....	.....	.....	.....	5
Cont. 8..	Vinca major	4.254	.....	.....	.....	.....	.....	.....	4
Exp. 9...	Vinca major*	6.560	.....	.....	.....	.....	.....	.....	15
Cont. 9..	Vinca major*	6.790	.....	.....	.....	.....	.....	.....	15
Exp. 10B	Vinca major	8.220	.....	1276	....	7.42	.2777	5.77	4
Cont. 10B	Vinca major	6.970	.....	1297	.....	6.56	.2141	5.09	4
Exp. 11..	Ricinus communis	43.860	.....	.....	.....	.....	27.07	.....	12
Cont. 11.	Ricinus communis	41.220	.....	.....	.....	.....	25.99	.....	12
Exp. 12..	Rubus occidentalis	.....	.....	.....	0.167	.....	1.216	.....	5
Cont. 12.	Rubus occidentalis	.....	.....	.....	0.147	.....	1.762	.....	5
Exp. 13..	Vicia Faba*	7.190	.....	.....	.....	.....	.....	.....	8
Cont. 13.	Vicia Faba*	8.090	.....	.....	.....	.....	.....	.....	7
Exp. 14..	Vicia Faba	24.910	.....	1630	.....	.....	22.75	....	7
Cont. 14.	Vicia Faba	20.590	.....	1419	.....	.....	21.00	.....	8
Exp. 15..	Lupinus albus	.....	.....	839	.....	.....	.9127	.....	5
Cont. 15.	Lupinus albus	.....	.....	755	.....	.....	.8000	.....	6

* See experiments for explanations of negative results.

xylem cells walls in *Sinapis alba* and *Vinca major* was also greater in the experimental stems than the controls.

Conclusion

The results of the foregoing experiments have convinced the writer conclusively that actively growing stems of the herbaceous plants investigated and of *Vinca major* respond to traction along their longitudinal axes, by increasing their breaking strength; also by an increased development of bast or of xylem, and in most cases by an

increase of both these mechanical tissues. The experimental evidence for *Rubus occidentalis* was too limited to be conclusive. The stems were already too old and mature when the experiment was started. The writer is convinced, however, by this experiment with *Rubus occidentalis*, that the tension used by WIEDERSHEIM (**29**) on pendant woody branches was not sufficient to send a stimulus past the stimulus threshold.

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THE MODIFIABILITY OF TRANSPIRATION IN YOUNG SEEDLINGS

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(WITH FIVE FIGURES)

The writer has often noticed the fact that young seedlings (e. g., of Cucurbita and Phaseolus) grown under bell glasses wilted almost at once when exposed to the dry air of a furnace-heated or steam-heated room. Gardeners all know that plants started in cold frames must be "hardened" by gradual exposure to the ordinary atmosphere, brought about by lifting slightly the sashes with which the cold frames are covered.

It appeared worth while to investigate quantitatively the rate of transpiration of these tender seedlings, grown in an extremely moist atmosphere, and the simplest possible case for study seemed to be that of annuals, for these cannot be supposed to have inherited a tendency to develop extreme adaptations to an abnormally moist atmosphere during part of the brief lifetime of the individual plant. Seedlings of the following species were grown in well-watered earth, some under glass cases with air-tight joints, and others in the free air of a furnace-heated room.

Cucumis sativus
Ipomoea purpurea
Lupinus albus
Mirabilis Jalapa
Nicotiana "Sanderae"
Oxalis corniculata
Phaseolus vulgaris
Salvia splendens
Sinapis alba

The temperature was on the average about the same for the covered and the uncovered plants; the former, of course, received a trifle less light than the latter. The moisture of the atmosphere about the leaves naturally differed greatly. Those in glass cases were in a nearly or often quite saturated atmosphere. Those in the free air of the room were in an atmosphere of which the relative humidity during the winter months ranged from 16 to 32 per cent., averaging less than 25 per cent. This is drier than the average summer atmosphere of an oasis in the Sahara. Some plants were also grown in a

greenhouse, where the relative humidity was usually not far from 60 per cent.[1]

In general the plants grown in nearly saturated air and in dry air or in that of moderate humidity presented considerable contrasts in their development.[2] Those from moist air were taller, more slender, longer-leaved, less hairy (if the plant were naturally pubescent), with

FIG. 1.—Ipomoea plants, moist-air form and dry-air form. $\times\frac{3}{4}$. Photographed by ROBERT CAMERON.

thinner, lighter-colored, more pliable, and more translucent leaves. In many cases the moist-air plants showed an accelerated development, producing more leaves in a given time and flowering earlier than those in drier air. Most of the exact measurements of relative

[1] The writer's thanks are due to Professor GEORGE L. GOODALE of Harvard University for the use of space in the greenhouses of the university.

[2] See WIESNER, J., Formänderungen von Pflanzen bei Cultur im absolut feuchten Raume und im Dunkeln. Ber. Deutsch. Bot. Gesells. 9:46–531. 1891.

development were made on plants of Phaseolus. In different lots of these, the moist-air individuals were 15 to 40 per cent. taller than those grown in drier air. The leaves of the former were sometimes as much as 80 per cent. longer than those of the latter, the difference being largely in the petioles. On the other hand, the diameter of the first internode above the cotyledons was (in plants grown in the greenhouse) 30 per cent. greater for those outside the moist glass cases. For house-grown plants the leaf thickness was 25 to 40 per cent. greater in the dry-air plants, and for those grown in the greenhouse

FIG. 2. Sinapis, entire stem and leaves, moist-air form. $\times\frac{1}{3}$.—FIG. 3. Sinapis, upper half of stem and leaves, dry-air form. $\times\frac{1}{3}$.

it was 25 per cent. greater than for those under glass cases. The most notable differences in growth of dry-air and moist-air individuals were shown by Ipomoea, in which the moist-air specimens were twining freely, when dry-air specimens of the same age had relatively short internodes and showed no tendency to twine (*fig. 1*). Mirabilis plants in nearly saturated air, by the time the second pair of true leaves had spread apart, were three times as tall as those grown in house air and much more slender. Sinapis, on the other hand, appears depauperate when grown in nearly saturated air, as shown in *figs. 2, 3*. The leaves of Sinapis, and of Cucumis also, in very moist air often show the less indented margin referred to by WIESNER.[3]

[3] WIESNER, J., Biologie der Pflanzen 65, 66. Wien. 1902.

The relative translucency of moist-air leaves and dry-air leaves was approximately measured in the case of Nicotiana and of Ipomoea by blue-printing them with various terms of exposure to sunlight, until an equal degree of blueness was obtained for the portions of paper covered by each kind of leaves.[4] The moist-air leaves of Ipomoea were found to be 3.5 times as translucent as the others, and those of Nicotiana 3 times. In the latter plant many of the first leaves grown in moist air stand nearly vertical, while those of the dry-air plants form approximate rosettes. This vertical growth of the moist-air leaves is exactly the reverse of the epinasty of the leaves of *Sempervivum tectorum* and *Oxalis floribunda* noted by WIESNER.[2] The differences in form and size between Nicotiana leaves and Ipomoea leaves grown under moist and under dry conditions are shown in *figs. 4, 5*.

The histology of the leaves studied did not differ nearly as much as it often does in sun plants and shade plants of the same species. In house-grown individuals of Phaseolus, leaves developed in dry air exceeded in thickness those in the glass cases by 25 to 33 per cent., the upper epidermis of the former was about 25 per cent. thicker, and the palisade layer was a little thicker. On the other hand, moist-air leaves of Sinapis were found to be a little thicker, and of Ipomoea sometimes 25 per cent. thicker, than leaves of these genera grown in dry air. As might have been expected, less notable differences were found between leaves grown in air nearly saturated with moisture and those grown in the moderately moist air of the greenhouse.

The behavior of moist-air leaves and dry-air leaves, on being deprived of a water supply and exposed to air at a temperature of about 21° C. and 25 per cent. relative humidity, differs greatly. If a shoot of each kind is cut and exposed to such air, in many cases (Brassica, Cucumis, Ipomoea, Oxalis, Phaseolus) wilting begins in from 0.5 to 2 minutes. Even if the shoots are cut under water and kept with the cut end always submerged, wilting is prompt and continuous. Shoots of Phaseolus were cut and laid in sunshine, in air of humidity probably below 25 per cent., at a temperature of 23°.3 C. One shoot was from the saturated air of the glass case, the other from

[4] This of course only measures translucency with reference to those rays which affect the blue-print paper

dry house air. Bits of the lower epidermis were peeled from the under surface of each kind of leaf at about 2-minute intervals and instantly placed in absolute alcohol to fix the stomata.[5] The operation was repeated on another day with fresh sets of leaves. It was found that in less than six minutes the stomata of the dry-air leaves were most of them closed to less than half their initial width, while

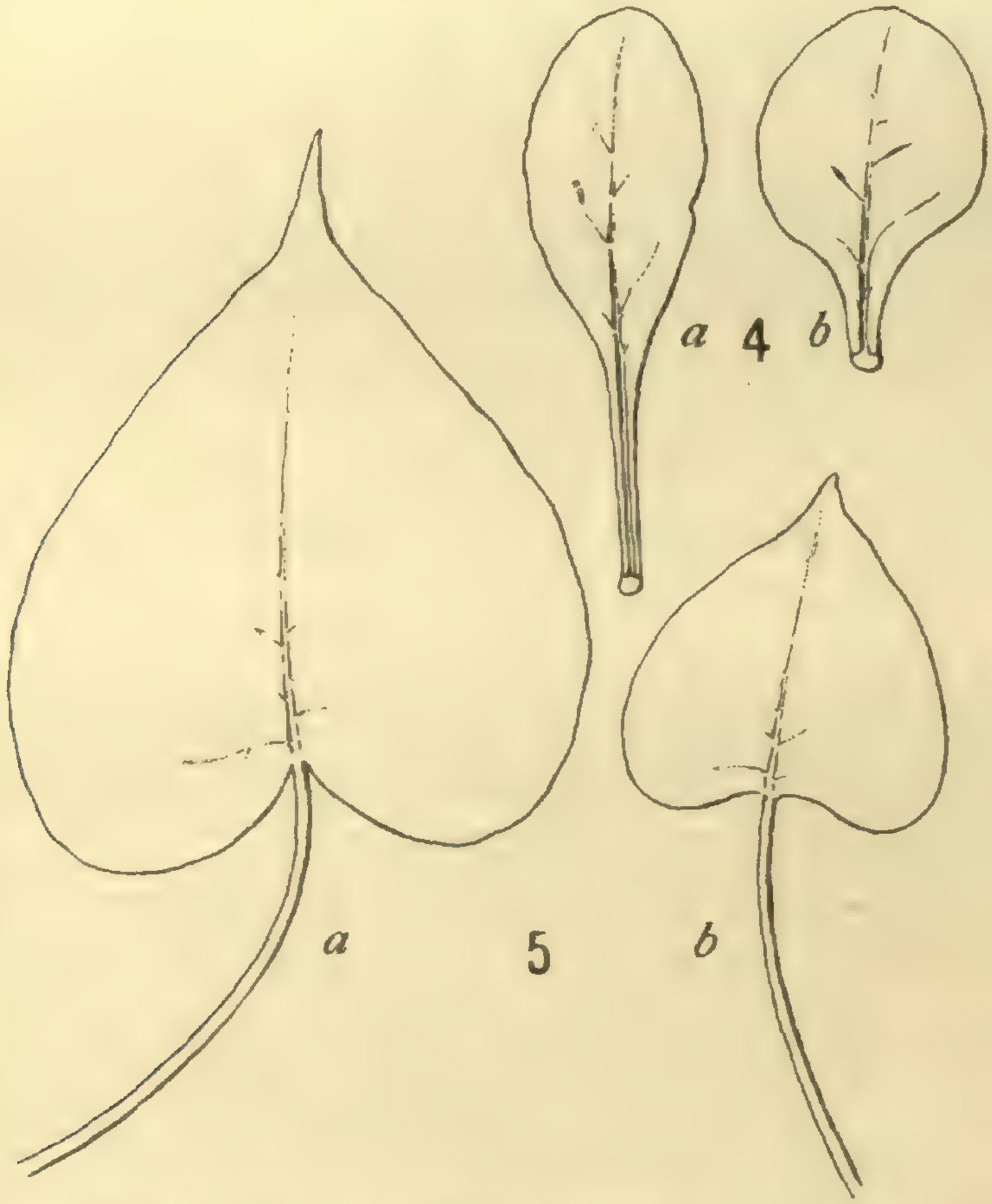

FIG. 4. Nicotiana leaves; *A*, moist-air form; *B*, dry-air form. ×½.—FIG. 5. Ipomoea leaves; *A*, moist-air form; *B*, dry-air form. ×½.

those of the moist-air leaves were but little affected. In about 15 minutes most of the stomata of the dry-air leaves were tightly closed, while most of those of the moist-air leaves still remained open. Apparently the speedy wilting of the moist-air leaves is due to two causes, the insufficient closure of the stomata and the relatively high permeability of the general surface of the epidermis to moisture.

[5] LLOYD, F. E., The physiology of stomata. Publ. 82, Carnegie Institution of Washington. 1908.

A curious kind of quick adaptation to dry conditions may sometimes be noted. Young leaves of Ipomoea, grown in nearly saturated air, were found to wilt in air of less than 30 per cent. relative humidity in about two minutes, though the stem was cut off under water and kept immersed in water. After being left for some hours in a saturated atmosphere until the wilting had entirely disappeared, the shoots were left, with the cut ends in water, in an atmosphere of less than 30 per cent. relative humidity for 48 hours without showing any signs of wilting. More than 90 per cent. of the stomata were at this time found to be perfectly closed.

The relative transpiration rate in diffuse light of moist-air and dry-air leaves of nearly all the kinds of seedlings grown was carefully determined. Attempts to make use of very slender (and therefore quick-reading) burettes as potometers were not successful. It was found too difficult to attach the soft and readily crushed stems of young seedlings to the burettes in such a way as to be sure to obviate leakage. All losses by transpiration were therefore estimated by weighing the shoots and the tubes of water which contained them on a balance sensitive to 5^{mg}. The relative humidity of the air in which the transpiration took place was measured by the sling psychrometer (sometimes twice) during each experiment. As might have been expected, the inequality of transpiration was found to be greatest in the case of fully developed leaves, half-grown ones showing less, though notable, differences. The values given below are for the ratio M/D, in which M is the transpiration of the moist-air leaf and D the transpiration of the dry-air leaf.

In discussing the results above given, it should be noted that a considerable range of values in the ratios obtained is almost unavoidable. In the first place we have to reckon with the great variability of transpiration in individuals of the same species grown under identical conditions. F. HABERLANDT[6] found in the case of rye plants that the transpiration per day varied (in round numbers) from 2 to 7^{gm} per square decimeter for different individuals. Also, if the transpiration were allowed to take place in a nearly saturated atmosphere (to prevent sudden wilting), the leaves would be under condi-

[6] HABERLANDT, F., Wissensch.-prakt. Untersuchungen auf dem Gebiete des Pflanzenbaues 2:146. Wien. 1877.

tions abnormal for the kinds of plants studied. If the atmosphere were to be made very dry, the leaves grown in very damp air would wilt inside of five minutes or less and die in a comparatively short time. In some cases it was found best to determine the transpiration of readily wilted leaves for a period of fifteen minutes and compare

TRANSPIRATION RATIOS

Kind of leaf	Rel. humid. during trans. period	Ratio M/D
Phaseolus	25	2.2
Phaseolus	63	2.5
Phaseolus	64	3.2
Lupinus	26	4.0
Lupinus	about 43	8.2
Lupinus	about 38	10.0
Lupinus	variable, probably over 40	1.9*
Mirabilis	36	3.9
Mirabilis	29	4.8
Ipomoea	26	7.4
Salvia	34	6.6
Salvia	..†	8.9
Sinapis	28	3.5
Cucumis	24	3.9*
Cucumis	34	9.3
Nicotiana	about 50	3.1

* Young leaves, only half grown or less.

† The moist-air leaves transpired on May 12 in air of 28 per cent. relative humidity, and the dry-air ones on May 13 in air of 34 per cent. relative humidity.

this with the (calculated) fifteen-minute loss of the dry-air leaf of the same species, allowed to transpire for one or two hours. *Oxalis corniculata* from saturated air became wholly wilted inside of one minute, on being removed from the glass case, so its transpiration ratio could not be determined. It would be interesting to compare the relative transpiration of the two kinds of leaves in extremely dry air minute by minute, but weighings on a balance delicate enough for this purpose could not be made with sufficient rapidity.

The conditions as regards relative humidity were intentionally varied a good deal, in order to show whether the transpiration ratios follow closely the changes in humidity. It is evident that in Phaseolus and Lupinus they do not.

While no experiments were made with a view of measuring the absolute rates of transpiration of the plants studied, all under the same conditions of temperature and humidity, it may be worth

while to give the values obtained for moist-air leaves of some of them.

TRANSPIRATION OF 100 SQ. CM. OF LEAF IN AN HOUR

Plant	Temperature °C	Rel. humid. per cent.	Transpiration in milligrams
Cucumis.....	20	34	1485
Lupinus.....	21.67	43	3596
Nicotiana.....	21.11	50 or less	1950
Phaseolus.....	26.11	25	1647
Sinapis.....	22.22	28	2135

Summarizing the results obtained from the transpiration measurements,[7] it may be said that:

1. As a result of being grown in a highly humid atmosphere, all the plants studied acquire a much greater than normal capacity for transpiration in a moderately dry atmosphere.

2. Different families and different genera of the same family vary greatly in their capacity to acquire by such culture a tendency to extremely rapid transpiration.

3. The transpiration ratios, for the same species, become notably greater as the leaf becomes fully developed.

4. The transpiration ratios are not necessarily greater when the relative humidity of the air, during the period when transpiration is measured, is very low than when it has a medium value.

CAMBRIDGE, MASS.

[7] Not nearly all of these results have been tabulated in the preceding pages.

A REMARKABLE AMANITA[1]

GEORGE F. ATKINSON

(WITH EIGHT FIGURES)

During the autumn of 1908 I received from Mrs. VIRGINIA GARLAND BALLEN, of Brookdale, Santa Cruz Co., Cal., a number of specimens of an Amanita which presents several remarkable peculiarities in its development and environic relations. For several years Mrs. BALLEN has observed this Amanita and has made a careful field study of the more salient features of its development. This account of the fungus is based on fresh specimens and photographs which she has sent me, and upon her notes and descriptions, which show a remarkable appreciation on her part of the important morphological characters, as well as of important features of development.

This plant grows in the mountain forests of California. It is among the largest species of Amanita, the cap being 10 to 22^{cm} in diameter, one of the larger ones, according to Mrs. BALLEN, being sufficient for a meal. It thus rivals in size the royal Amanita of Europe, which it surpasses in robustness, though not possessing its rich orange-yellow color, and not attaining the height of the larger specimens of that species. It is interesting to note that the stocky character of this plant with its short stem is probably an expression of one of its environic and seasonal relations. It occurs in the high Sierras and in the Coast Range. Probably the entire summer season is needed for the growth and extension of the mycelium in the forest mold, so that the huge fruit bodies are developed in late autumn and early spring. While we have as yet no information bearing on the time of origin of the fundament of the fruit bodies, it is likely that all of them are formed during the summer and late autumn, and that the second crop, which appears early in the spring, is composed of plants which have lived through the winter in a partially developed condition. The autumn crop ceases about the last of December, while the spring crop begins about the middle of March.

[1] Contribution from the Department of Botany, Cornell University, No. 135.

In the high Sierras, where it is colder, the plant is well protected from frost injury, since it rarely if ever appears above the carpet of leaves covering the forest floor. Here it grows about the pines and firs. It has here almost become a subterranean fungus, a remarkable

FIG. 1.—Partly expanded plant, showing calyptra of volva closely adhering to the pileus, the veil with the floccose remnants of the fundamental tissue between it and the stem, and the limb of the volva at base of stem.

thing for an Amanita. Thus it is difficult to find, the only evidence of its presence being the mounds of conifer needles which the hidden plants raise above them. Uncovering these and removing the plant there is a large cone-shaped hole in the soil made by the pressure of the very thick stem and cap. They are often found in bitter cold

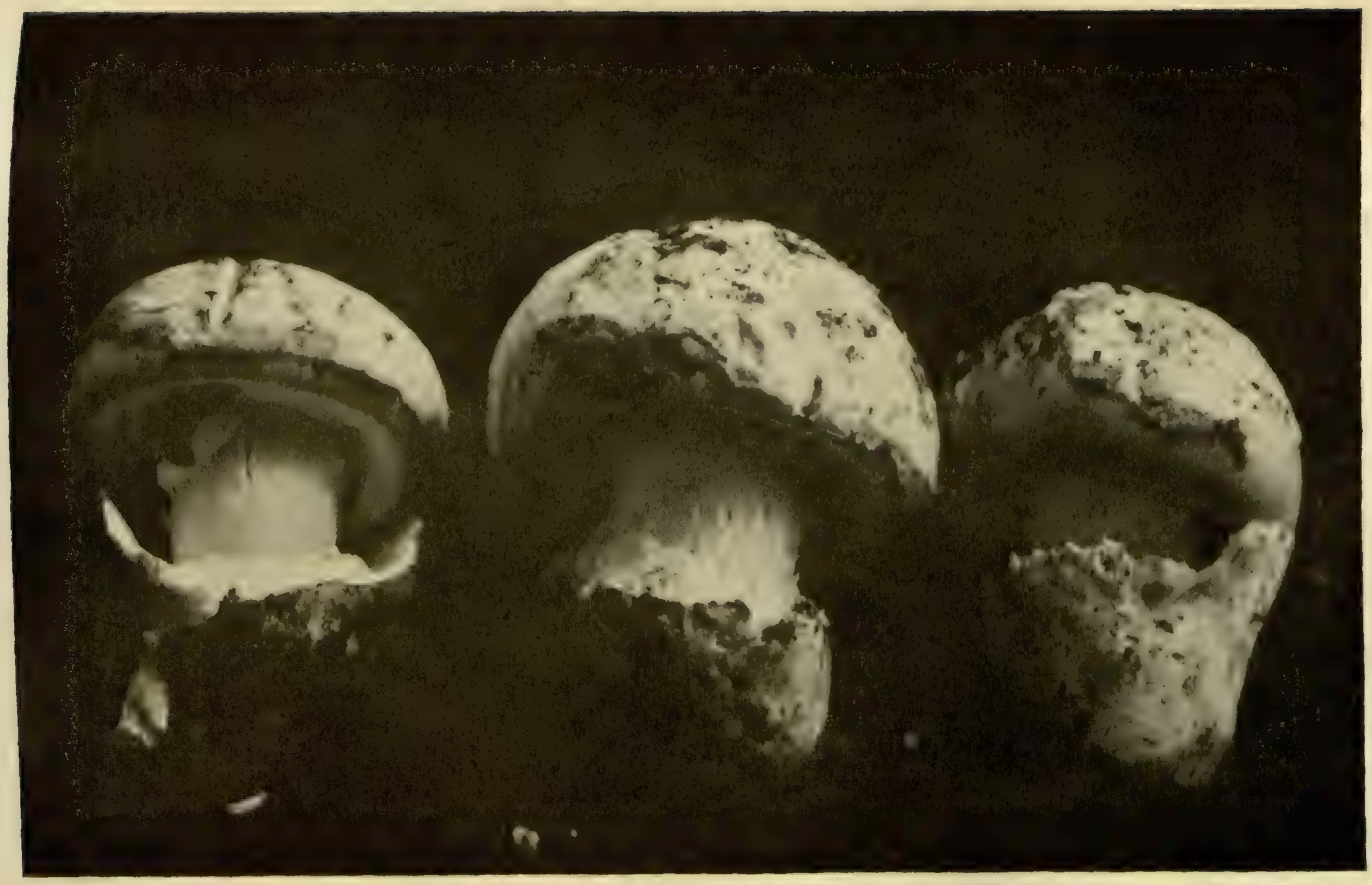

FIG. 2.—Three plants showing circumscissile dehiscence of the volva; the plant at the left with the delicate veil torn and (in front) the inner collar of the volva.

weather, when the ground is covered with snow, but are so well protected by the covering of needles and snow that they are perfectly fresh and uninjured, while a few hours' exposure to the air results in their being frozen. In the Coast Range they are smaller and not so

FIG. 3.—Partly expanded plant, showing veil attached at apex of stem and supported in a divergent position by the mass of loose cottony remnants of the fundamental tissue lying between the veil and stem.

bright-colored, and are found around the madroñas, chestnuts, oaks, pines, and spruces. Mrs. BALLEN has never found them around the redwoods. The spring crop is also paler than the autumn crop. In the mountains about Brookdale, Santa Cruz Co., they appear above the ground.

The pileus is a maize-yellow in its bright-colored forms and varies to a pale straw color or Naples yellow (R.) in the vernal forms. The gills are at first white and later become tinged with the same color. The stem and inner veil or annulus are also tinged with pale straw

FIG. 4.—Section of plant showing pendent veil and loose cottony fundamental tissue between it and the stem.

color. The volva is thick, stout, and white, though in age it becomes more or less soiled and tinged with yellowish brown. The pileus is broadly rounded in the young plants, becoming broadly convex to plane, or in old plants the margin may become elevated, thus giving the pileus a depressed appearance.

Very little of the surface of the pileus is visible, since all except a narrow marginal portion is covered with the calyptra of the volva, which forms a thick, white, tough skin or covering, closely and tightly fitting the cuticle of the pileus. In cutting through the calyptra and pileus the line of junction can be seen, and in fresh plants this line of division is brought out distinctly because of the pale-yellow cuticle of the pileus. This calyptra, covering the larger part of the pileus like

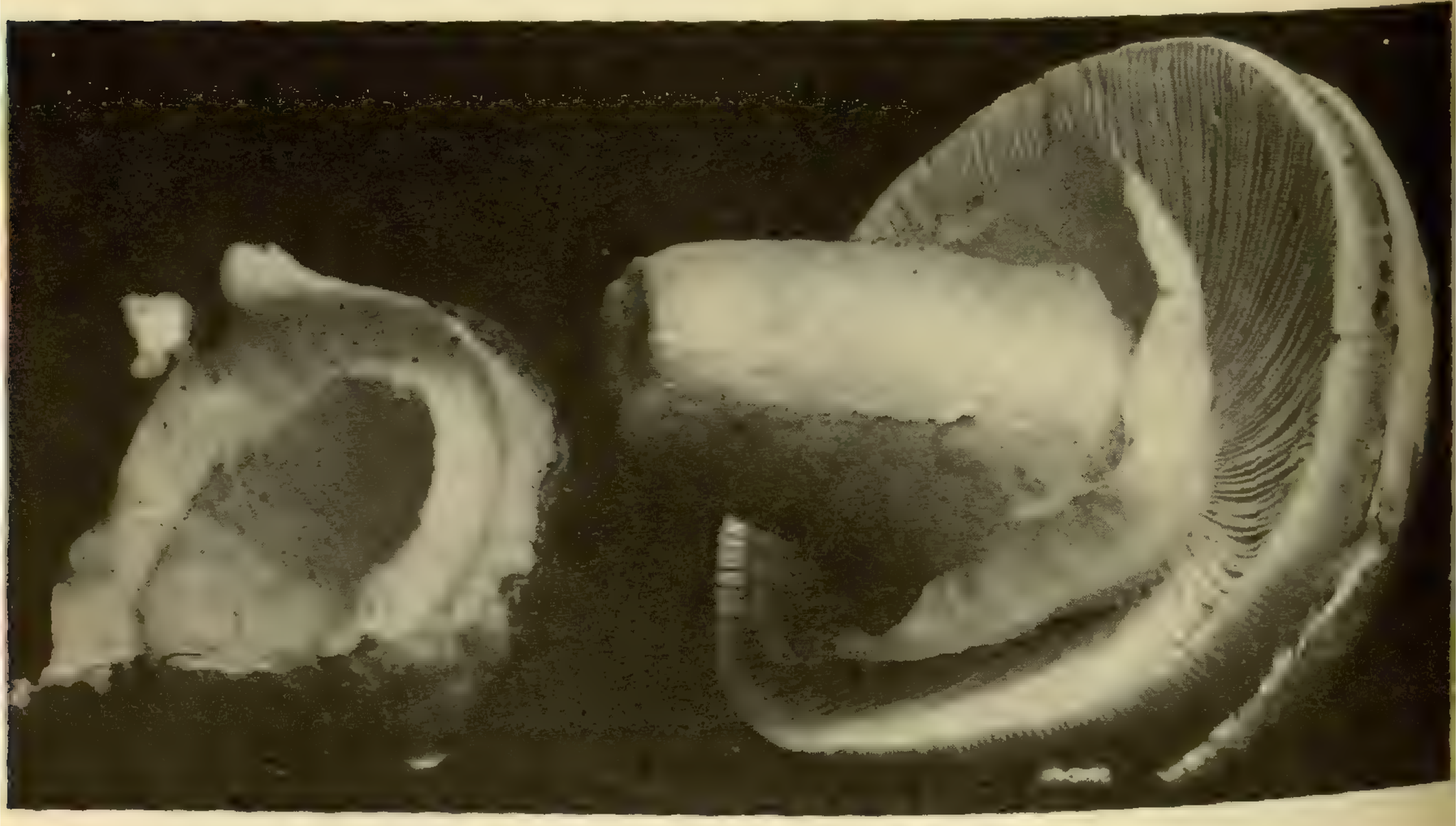

FIG. 5.—The stem separating from the volva; note inner collar of the volva, striate margin of the pileus, and very distinct thick calyptra.

a closely fitting cap, is often unbroken; but in large, well-expanded plants, it is often cracked into irregular large areas, as in *fig. 6*, showing the pileus between.

The inner veil is very thin and fragile, and disappears soon after the opening of the plant. When the plant is expanded the annulus is attached to the extreme upper end of the stem at the point of junction of the gills and stem, for the gills are adnexed, as in some other species of Amanita, and the stem is not readily separable from the pileus. The plant is very fleshy, is often attacked by larvae, and readily decays, so that transport of mature or nearly mature specimens is difficult.

In the button stage they sometimes carry and keep well for several days.

The inner veil is slightly connected with the edges of the gills and thus the expansion of the pileus produces a tension which tears the veil from the surface of the stem, with which it is also connected by

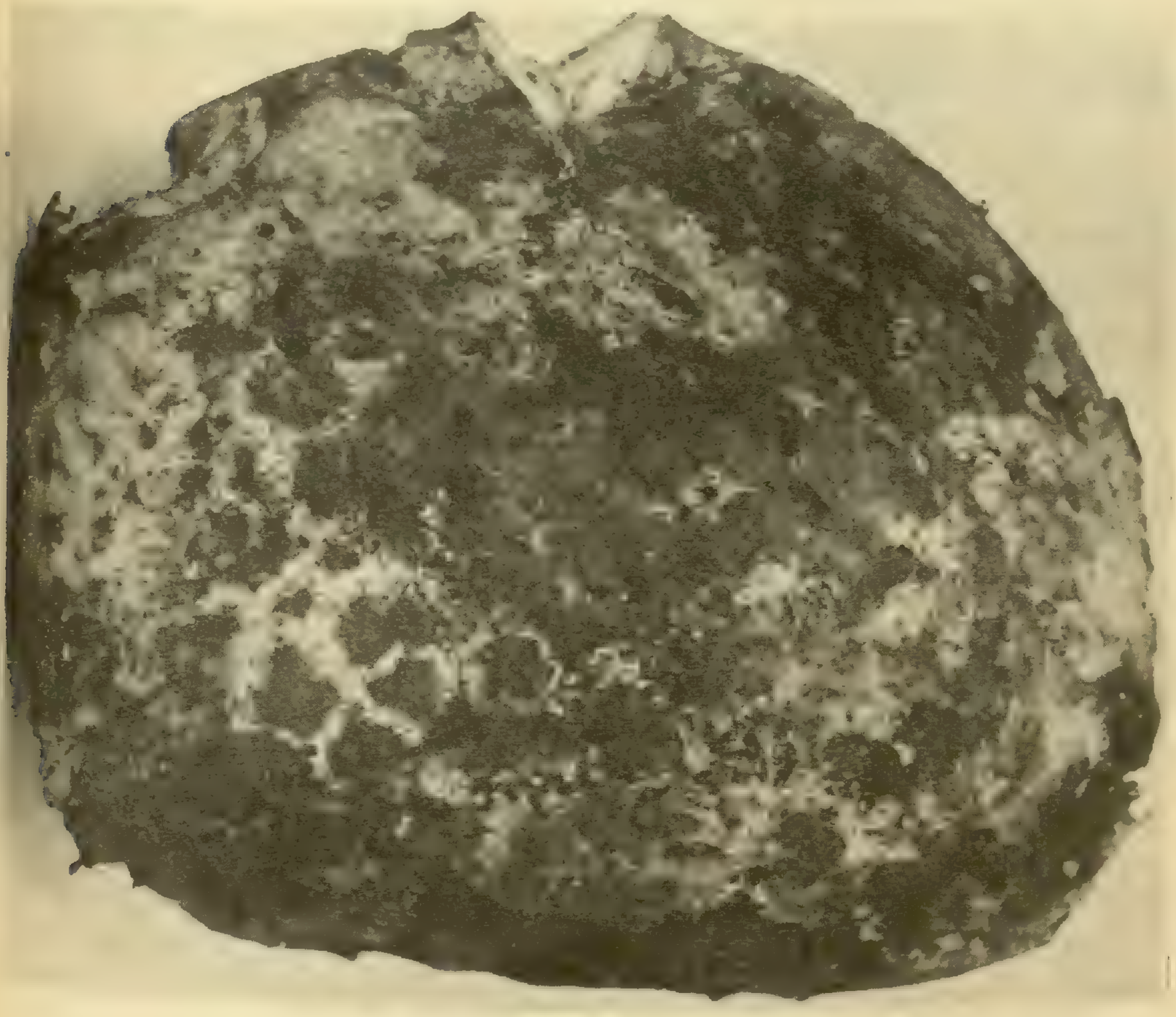

FIG. 6.—Surface of pileus, showing calyptra torn into large patches, which remain tightly adherent to the pileus.

remnants of the fundamental tissue. This tissue, lying between the veil and the stem, is very loose and floccose, so that it is torn into a delicate cottony mass, which often supports the veil in a divergent position as it hangs from the apex of the stem (*figs. 3, 4*). The veil itself is very delicate in texture, and Mrs. BALLEN says that it "seems to melt away" soon after the expansion of the plant. This indicates that it is not well differentiated from the fundamental tissue. In

buttons which were shipped to me, and which opened in transit or after reaching here, this inner veil did not separate from the stem, but remained as fundamental tissue clothing the stem (*fig. 7*, where the outline of the cortex of the stem is shown within).

The stem is stout and comparatively short. The volva is circumscissile, but the lower half, which remains attached to the base of the stem, is large, with an ample, stout, free limb forming a large sac-like structure resembling that of the Amanitas with apical dehiscence, though the edge is more even. Within the basal portion of the volva and surrounding the stem there is often present a narrow collar or secondary sheath, the origin and nature of which, to my knowledge, have never been carefully described in any Amanita, for it is usually overlooked.

This inner collar or secondary sheath I have studied carefully in *Amanita caesarea* of Europe, while studying the higher fungi in the Jura Mountains of France, from specimens collected at Besançon and Arbois, in September, 1905. PLOWRIGHT[2] has called attention to a similar inner collar in specimens of *Amanitopsis spadicea*, and proposed to employ it in separating this species from *A. livida*, the two being usually brought together under this name, but he offered no suggestion as to its significance or origin. I have observed it and studied its origin also in *Amanitopsis livida* Richon & Roze and do not think that much specific importance can be attached to it, since it varies so in strength in different specimens and is often so obscure when it remains, as it sometimes does, closely applied against the base of the stem. Great credit, however, is due to Mrs. BALLEN for having made such careful observations on the presence and nature of this interesting structure in the California Amanita, the more so since there is no published description of such a structure, and her observations, though later than mine on *Amanita caesarea* and *Amanitopsis livida*, were entirely independent of them, and made before she had called my attention to the existence of this species.

Longitudinal sections of the young plants when in the more advanced "egg" stage show all of the principal parts well formed. The pileus and stem when cut or bruised often turn a pale straw

[2] Trans. Brit. Mycol. Soc. **1897–8:**40. See ATKINSON, Mushrooms, edible, poisonous, etc. 75. 1st ed. 1900, Ithaca. 2d ed. 1901, Ithaca, and 1903, New York.

FIG. 7.—Plants photographed after transit from California to Ithaca, N. Y., showing dehiscence; middle plant in section, showing very distinctly the thickness of the calyptra; inner veil remaining attached to the stem and continuous below with the tissue that forms the collar on the inside of the volva, indicating that all of the tissue which lies between the stem and the gills is fundamental tissue.

yellow on exposure of the cut surface to the air. In such sections of young plants therefore the pileus is clearly marked off from the surrounding volva, and the wall of the stem is likewise marked off distinctly from the surrounding fundamental tissue. The gills are also well outlined. The slightly convex outline of their edge does not permit them to lie against the surface of the stem, and their lower or outer ends curve away from it in the young stage. This space is filled with fundamental tissue, and as the plant expands it is left free from

FIG. 8.—Photomicrograph of spores with Zeiss ocular 18, objective 3^{mm}; object 370^{mm} from plate-holder.

the stem and gills, but attached to the inner side of the base of the volva as a collar around the stem. When the plants become quite mature or old, there are tissue changes at the base of the stem inside of this collar which permits the stem to be very easily separated from the volva. By this time the free limb of the volva has recurved more or less, leaving the volva in the shape of a saucer with a recurved edge, and an inner collar. At this stage, if one takes hold of the cap to lift the plant, the stem is freed from the volva cup, leaving this saucer-shaped structure in the ground. *Fig. 5* is from a photograph of this stage, showing this saucer-like structure of the volva with its inner collar, and the freed stem at one side. In dry weather this separation of the stem from the volva does not take place.

I have proposed the name *Amanita calyptroderma* for this plant,

because the calyptra of the volva fits like a skin over the center of the pileus. Although a technical description has been published elsewhere,[3] it may be well to add a diagnosis here.

AMANITA CALYPTRODERMA Atkinson & Ballen.[4]—Plants 10–15^{cm} high, pileus 10–22^{cm} broad, stem 2–4^{cm} stout. Pileus maize-yellow to pale chrome-yellow, gills white, then pale maize to cream color, annulus and stem pale maize to cream color, volva white. Pileus stout, fleshy, convex to expanded and even depressed in age, margin striate, slightly viscid when moist, larger part of pileus covered with the tough thick calyptra of the volva which fits closely like a skin, the margin free, while in age in the larger plants the calyptra of the volva is cracked into rather large areas by the expansion of the pileus. Gills broad, adnexed, edge more or less floccose. Basidia 4-spored. Spores oval to elliptical, smooth, coarsely granular, 8–12×7–8 μ. Annulus very thin, membranaceous, superior, hanging from the extreme apex of the stem, soon disappearing. Stem hollow, with loose cottony threads, even in the smaller plants, tapering upward in the larger ones, or smaller at base, surface floccose. Volva white, thick, circumscissile, in dehiscence the upper part remaining on the center of the pileus, lower portion of limb very prominent, 2–4^{cm} long, sometimes appressed on the stem, but usually distinctly divergent and in age recurved, often with a distinct inner collar near the base, in age the stem often separating from the volva, leaving the latter as a saucer-shaped structure with its inner collar in the ground.—Mountain forests, California, in late autumn and early spring, *Mrs. Virginia Garland Ballen.* Herb., Cornell Univ., no. 22620.

[3] Science N. S. **29**: 944. 1909.

[4] Closely related to *Amanita calyptrata* Peck, Bull. Torr. Bot. Club **27**: 14. 1909, but differs in color and other characters.

UNDESCRIBED PLANTS FROM GUATEMALA AND OTHER CENTRAL AMERICAN REPUBLICS. XXXII[1]

JOHN DONNELL SMITH

Pithecolobium (§SAMANEA Benth.; Ser. *Carnosae* Benth.) **catenatum** Donn. Sm.—Folia ampla, pinnis unijugis, foliolis 3–5-jugis obovato-oblongis vel -ellipticis obtuse cuspidatis basi acutis, stipulis uti glandulae interpetiolulares obsoletis. Pedunculi axillares longissimi. Legumen lineare longissimum moniliforme polyspermum bivalve, loculamentis atque seminibus ellipsoideis.

Frutex, ramis petiolis pedunculis ferrugineo-puberulis. Petiolus communis 4–8^{cm} longus, pinnarum erecto-patentium rhachi 12–22^{cm} longa, foliolis chartaceis praeter costam utrinque puberulam glabris subtus pallidioribus in eodem jugo inaequalibus per paria deorsum decrescentibus, supremis 11–16^{cm} longis 5–7·5^{cm} latis, infimis 3–9^{cm} longis 2–4·5^{cm} latis, nervis lateralibus utrinque 6–7, venulis subtus minutissime reticulatis, petiolulis 2–3^{mm} longis. Pedunculi 8–18^{cm} longi, floribus sessilibus. Legumina crasse coriacea rubiginoso-subvelutina addito stipite 12–22^{mm} longo 24–26^{cm} longa epulposa demum dehiscentia inter semina arcte constricta, loculamentis 15–17^{mm} longis 9–11^{mm} crassis leviter compressis, isthmo infimo interdum 12–14^{mm} longo, ceteris 2–4^{mm} longis, seminibus 11–12 circiter 13–15^{mm} longis 8–10^{mm} latis atrocoloribus, hilo subapicali.—Florum tantum reliquiae visae. Ad *P. sophorocarpum* Benth. legumine semine embryone accedens foliis longe distat.

In silvis profundis ad praedium *Suerre* vocatum, Llanuras de Santa Clara, Comarca de Limón, Costa Rica, alt. 300^{m}, Febr. 1896, *John Donnell Smith*, n. 6479 ex Pl. Guat. etc. quas ed. Donn. Sm.

Appunia guatemalensis Donn. Sm.—Glabra. Stipulae breviter vaginantes bicuspidatae. Folia subsessilia magna elliptico- vel obovato-oblonga utrinque acuminata. Capitulum singulum pauci- et laxi-florum, bracteis ovario paulo longioribus glandulis 1–3 cuspidatis. Calyx campanulatus truncatus basi glanduligerus quam corolla octies brevior.

Frutex. Stipulae coriaceae acuminato-triangulares 3^{mm} longae, cuspidibus minutis. Folia subcoriacea utraque pagina nitentia 13–16^{cm} longa medio vel paulo supra medium 5–7^{cm} lata, omnia opposita, nervis lateralibus utrinque 5–6, petiolis 2–3^{mm} longis. Pedunculi axillares 3·5–4^{cm} longi, capitulo circiter 7-floro absque corollis 5–6^{mm} longo atque lato, bracteis acuminato-ovatis 1·5^{mm}

[1] Continued from BOT. GAZETTE **47**: 262. 1909.

longis, floribus heterogoneis (brevistylis solum visis). Calycis 2^{mm} longi in sicco nigricantis tubus hemisphaericus limbum aequans, glandulis linearibus paucis. Corolla 16^{mm} longa ad 2^{mm} supra basin staminigera. Antherae subsessiles 3^{mm} longae. Discus 0.5^{mm} altus. Ovarium 1^{mm} longum, stylo uti stigma pyramidale 1^{mm} longo. Bacca ignota.

In silva prope pagam maritimam *Livingston*, Depart. Livingston, Guatemala, Jun. 1906, *H. von Tuerckheim* (n. II. 1230).

Palicourea (§Crocothyrsus Griseb.; Ser. *Croceae* Muell. Arg.) **leucantha** Donn. Sm.—Glabra. Stipulae vaginam amplam laxam truncatam subaequantes. Folia elliptico-oblonga utrinque acuminata. Thyrsus elongatus, axibus angulosis, bracteis bracteolisque majusculis. Calycis limbus alte partitus ovario 2–4-plo longior. Corolla alba ad $\frac{1}{4}$ altitudinis paleaceo-annuligera.

Internodia obtuse tetragona. Stipulae late sejunctae lineari-lanceolatae $4–5^{mm}$ longae. Folia subtus minute puberula $14–17^{cm}$ longa medio $4–7^{cm}$ lata, petiolis $1.5–3^{cm}$ longis. Thyrsus saepius foliis reductis fultus $13–18^{cm}$ longus supra basin $5–7^{cm}$ latus, ramis infimis arrectis $5–6^{cm}$ longis, bracteis bracteolisque lineari-lanceolatis $4–9^{mm}$ longis, cymis dichotomis, pedicellis $3–9^{mm}$ longis. Calycis lobi inaequales oblongo-ovati $2–4^{mm}$ longi trinervi. Corolla cylindrica computatis lobis 2^{mm} longis $15–16^{mm}$ longa margine virescens paleis niveis annuligera ad $\frac{3}{4}$ altitudinis staminigera. Antherae medio affixae 2^{mm} longae filamenta aequantes apice exsertae. Discus hemisphericus 1.5^{mm} altus. Ovarium cylindricum $1–1.5^{mm}$ longum atque crassum biloculare, stylo 7^{mm} longo, lobis 1.5^{mm} longis. Bacca deficit.—*P. mexicanae* Benth. affinis.

In silva montana ad viam inter Cobán et Tactic, Depart. Alta Verapaz, Guatemala, alt. 1800^{m}, Mart. 1903, *H. von Tuerckheim*, n. 8400 ex Pl. Guat. etc. quas ed. Donn. Sm.—In monte prope Cobán, Depart. Alta Verapaz, Guatemala, alt. 1650^{m}, Jun. 1908, *H. von Tuerckheim* (n. II. 2282).

Parathesis microcalyx Donn. Sm.—Folia lanceolato-obovata cuspidato-acuminata in petiolum attenuata integra supra glabra subtus glabrescentia. Panicula terminalis foliis superata. Calyx brevis, lobis deltoideis tubum aequantibus. Corolla staminibus bis longior. Ovarium apice pubescens totum post anthesin calyce aequilongo arcte cinctum depresso-globosum.

Folia recentiora subtus cum ramulis petiolis panicula floribus ferrugineo-pubescentia, provectiora glabra erga lucem inspecta punctulis et lineis pellucida, saltem superiora (solum visa) $9–12.5^{cm}$ longa $3.5–4^{cm}$ lata, petiolis $1–1.5^{cm}$ longis. Panicula $6–7^{cm}$ longa, floribus ad apicem versus ramorum secundariorum corymbosis, pedicellis minute lineari-bracteolatis $1.5–3^{mm}$ longis. Calyx 1^{mm} longus immaculatus. Corollae tubus 0.5^{mm} longus, laciniae lineari-lanceolatae 3^{mm}

longae acutae lineato-maculatae intus glabrae. Antherae ovatae 1^{mm} longae area dorsali atra deltoidea epunctatae filamentis bis longiores. Ovarium corolla jam delapsa 1^{mm} longum paulo latius quam longius, stylo 2.5^{mm} longo. Fructus desideratur.—*P. serrulatae* Mez proxima.

In fruticetis secus rivulum ad fundum *La Colombiana* dictum, Llanuras de Santa Clara, Comarca de Limón, Costa Rica, alt. 200^{m}, Jun. 1899, *H. Pittier*, n. 7591 ex Pl. Guat. etc. quas ed. Donn. Sm. (n. 13410 herb. nat. Cost.).

Gonolobus (§MONOSTEMMA K. Schum.) **leianthus** Donn. Sm.—Folia longe petiolata sparsim pilosiuscula ovato-oblonga acuminata sinu profundo rotundato cordata. Pedunculi biflori et pedicelli graciles, floribus glabris inter maximos. Segmenta corollina lanceolato-oblonga parum reticulata calycinis ovatis sesquilongiora. Corona tenuiter annularis subintegra gynostegio brevissimo adnata.

Frutex volubilis, ramis petiolisque patenter pilosis vel glabrescentibus, pedunculis pedicellisque glabris. Folia 9–12^{cm} longa 3.5–5^{cm} lata supra paene glabra subtus setulis minimis aspersa, petiolis 6–8^{cm} longis. Pedunculi 2–4^{cm} longi, pedicellis inaequalibus 3–5^{cm} longis, floribus 5^{cm}-diametralibus. Calycis segmenta paene sejuncta late imbricata herbacea 13^{mm} longa 5^{mm} lata acuminata. Corollae alte fidae segmenta 2^{cm} longa 7–9^{mm} lata acute elongata carnulosa in sicco badia erga lucem inspecta reticulata. Corona callis contiguis minutis multidenticulata mediantibus carinis tenuibus gynostegio connexa annulo membranaceo pubescente corollae adnato comitata. Gynostegium vix 1^{mm} altum 6^{mm}-diametrale.—*G. macrantho* Kunze proximus.

Cubilquitz, Depart. Alta Verapaz, Guatemala, alt. 350^{m}, Oct. 1901, *H. von Tuerckheim*, n. 8243 ex Pl. Guat. etc. quas. ed. Donn. Sm.

Trichostelma oblongifolium Donn. Sm.—Folia lanceolato-oblonga cuspidato-acuminata basi acuta vel obtusiuscula. Cymae sessiles. Segmenta corollina calycinis lanceolatis bis longiora ovato-lanceolata cymbiformia apice cucullato recurva. Corona exterior parce longeque ciliata, interior breviter cupularis vix lobata. Gynostegium breve appendicula paulo longius.

Suffruticosum volubile patenter pilosum. Folia discoloria utrinque pilis bulbosis aspersa 8–11^{cm} longa medio 3–5^{cm} lata, nervis subtus fusco-villosis, petiolis 1–2^{cm} longis. Pedicelli 3–5-fastigiati 1.5–3.5^{cm} longi, floribus extus hirsutis. Calycis partiti segmenta 4^{mm} longa reflexa sinubus uniglandulosa. Corolla nervosa 10^{mm} longa alte fissa. Corona exterior annularis hyalina, interior carnulosa corollae semiadnata a gynostegio libera anguste undulato-lobata margine extus lineari-rugosa. Appendicula a gynostegio pendula oblongo-bilobata 1.5^{mm} longa lobis divaricatis 3^{mm} lata. Pollinia pendula oblongo-elliptica caudiculis paulo longiora. Folliculi desunt.—A specie hactenus unica, *T. ciliato* Baill. (*Fimbristemma calycosa* Donn. Sm.), foliis atque floribus optime distinctum.

In fruticetis ad Panzal, Depart. Baja Verapaz, Guatemala, alt. 1000^{m}, Apr. 1907, *H. von Tuerckheim* (n. II. 1747).

Solanum (§ LEIODENDRA Dun.) **Rovirosanum** Donn. Sm.—Folia gemina parum inaequimagna elliptica vel obovato-elliptica bis longiora quam latiora in Sectione maxima sursum in angulum acutum vel obtusum desinentia in petiolum brevem plus minus attenuata, nervis lateralibus utrinsecus 6–7 ad axillas nudis. Pedicelli cymoso-fasciculati numerosi graciles, floriferi cernui, fructiferi erecti.

Frutex omnibus in partibus glaber. Folia pergamentacea concoloria nitida 19–30^{cm} longa 9–14·5^{cm} lata, altero circiter quinta parte minore, petiolis 8–18^{mm} longis. Pedunculi 10–24^{mm} longi nonnunquam furcati, pedicellis floriferis 6^{mm} longis, fructiferis 11^{mm} longis. Calyx obpyramidalis 2·5^{mm} longus, lobis tubo bis brevioribus rotundatis calloso-apiculatis. Corollae tubus 1^{mm} longus, laciniae lanceolatae 6–7^{mm} longae. Antherae conniventes oblongae 3^{mm} longae 1^{mm} latae nigricantes ad apicem luteum poris anticis amplis ellipticis dehiscentes, filamentis complanatis 1^{mm} longis. Ovarium ovoideum, stylo 5^{mm} longo. Bacca nondum satis matura calyce accrescente cincta globosa 7^{mm}-diametralis nigra.

Cubilquitz, Depart. Alta Verapaz, Guatemala, alt. 350^{m}, Aug. 1904, *H. von Tuerckheim*, n. 8716 ex Pl. Guat. etc. quas ed. Donn. Sm.; Jul. 1907, *H. von Tuerckheim* (n. II, 1888).—Eandem plantam collegit *José N. Rovirosa* in Mexico ad Mayito, Estado de Tabasco, Jul. 1889, et sub numero 544 distribuit.

Athenaea cernua Donn. Sm.—Glandulari-puberula. Folia glabrescentia plerumque solitaria ovata cuspidato-acuminata basi cuneata vel rotundata. Pedunculi axillares vel dichotomales solitarii, flore atque fructu cernuis. Calycis lobi elongato-triangulares sub anthesi tubo paulo longiores. Corollae limbus campanulatus triente lobatus, lobi deltoidei. Bacca coccinea.

Herbacea, ramis dichotomis sulcatis et petiolis glandulari-puberulis. Folia membranacea punctulata nervis et margine puberula 4–7·5^{cm} longa 2·5–4^{cm} lata integra interdum gemina, altero consimili triente minore, petiolis 1·5–3^{cm} longis. Pedunculi 2·5–3·2^{cm} longi subglabri, floribus 7^{mm} longis. Calyx glandulari-puberulus 3·5^{mm} longus, lobis sub anthesi 2^{mm} longis. Corollae luteae tubus cylindricus 1^{mm} longus, limbus abrupte ampliatus basi staminigerus, lobi 2–2·5^{mm} longi ciliolati. Antherae ovales 2^{mm} longae 1^{mm} latae filamentis subaequilongae. Ovarium ovoideum 1·5^{mm} longum, stylo 3·5^{mm} longo. Bacca globosa 8^{mm}-diametralis calycis aucti lobis 3·5^{mm} longis obvelata, seminibus fuscis scrobiculatis.

In silva ad praedium *Sasts*, Depart. Alta Verapaz, Guatemala, alt. 900^{m}, Maj. 1908, *H. von Tuerckheim* (n. II. 2245).

Brachistus ceratocalycius Donn. Sm.—Glabrescens. Folia lanceolata e medio utrinque acuteque angustata plerumque solitaria,

floralia gemina homomorpha subaequimagna. Pedunculi singuli vel 2–4-ni. Calyx medio infra marginem truncatam scariosam tuberculis 10 appendiculatus. Corolla infundibularis paulo ultra medium lobata. Antherae ellipticae filamentis altero tanto longiores.

Frutex, ramulis novellis furfuraceis. Folia 7–10^{cm} longa 1.7–2.5^{cm} lata supra primum puberula mox utrinque glabra, nervis lateralibus utrinsecus 5–6, venis transversis parallelis remotis parum manifestis, petiolis 5–12^{mm} longis et pedunculis calycibusque puberulis. Pedunculi 15–31^{mm} longi erecti. Calyx hemisphaericus 3.5^{mm} altus, margine scariosa 1^{mm} lata, tuberculis squarrosis subconicis. Corolla violacea (cl. repertor in scheda), 16–17^{mm} longa in alabastro griseo-pubescens, lobis lanceolatis ad apicem versus et ad margines pubescentibus. Antherae 5^{mm} longae 2.5^{mm} latae apiculatae margine exteriore dehiscentes. Ovarium ovoideum 2.5^{mm} longum in stylum aequilongum attenuatum. Bacca ignota.

In silva montana prope Cobán, Depart. Alta Verapaz, Guatemala, alt. 1600^{m}, Jan. 1908, *H. von Tuerckheim* (n. II. 2060).

Ruellia (§ DIPTERACANTHUS Baill.) **pygmaea** Donn. Sm.—Folia approximata minima oblongo-ovata acuminata basi acuta integra supra paleaceo-pilosa subtus nervis ferrugineo-strigillosa. Flores ad axillas oppositas foliorum superiorum subsessiles, bracteis linearibus calyce paulo longioribus corolla 5–6-plo brevioribus. Capsula puberula 4-sperma infra medium contracta et compressa.

Caules e rhizomate longe repente foliis abortivis munito passim ascendentes 6–8^{cm} longi simplices et petioli ferrugineo-strigillosi. Folia internodiis longiora superne imbricantia 13–25^{mm} longa 7–10^{mm} lata supra pilis longis articulatis aspersa utrinque lineolata, nervis lateralibus utrinsecus 4, petiolis 2–3^{mm} longis. Bracteae 4–5^{mm} longae paleaceo-pilosae. Calycis puberuli tubus 1.5^{mm} altus, segmenta linearia 2–2.5^{mm} longa. Corolla extus puberula 24^{mm} longa supra basin cylindricam 8^{mm} longam sensim et aequaliter ampliata faucibus 5^{mm} lata, lobis late rotundatis 3^{mm} longis. Stamina bene inclusa, antheris 2^{mm} longis. Capsula oblongo-ellipsoidea 8–9^{mm} longa acuminata sulcata, parte solida 4^{mm} longa, seminibus orbiculatis 2^{mm}-diametralibus margine cano-pubescentibus.—*R. humifusae* Hemsl. proxima.

Hacoc prope Cubilquitz, Depart. Alta Verapaz, Guatemala, alt. 600^{m}, Maj. 1904, *H. von Tuerckheim*, n. 8725 ex Pl. Guat. etc. quas ed. Donn. Sm.

Ruellia (§ PHYSIRUELLIA Lindau; Ser. *Eglandulosae* Lindau) **guatemalensis** Donn. Sm.—Folia glabra ovata vel lanceolato-ovata acuminata basi angustata vel rotundata margine undulata. Flores subsessiles 1–4-ni axillares vel in cymis axillaribus terminales et dichotomales, cymis folia superantibus, axibus elongatis, bracteis

longissimis. Corolla usque ad medium cylindrica tum ventricoso-infundibularis.

Frutex, ramis obtuse tetragonis minute cystolithigeris, angulis et nodis puberulis. Folia chartacea utrinque praesertim supra lineolata 9.5–10.5^{cm} longa 3.5–4^{cm} lata, vel superiora magis ovata 5^{cm} longa 2.5^{cm} lata, nervis lateralibus utrinsecus 4–5, petiolis puberulis canaliculatis amplexicaulibus 10–18^{mm} longis. Cymae nondum satis evolutae addito pedunculo 5–8^{cm} longo 10–14^{cm} longae puberulae parce furcatae pauciflorae, axibus erecto-patentibus 1.5–3.5^{cm} longis, bracteis linearibus 2–3^{cm} longis, bracteolis lanceolatis 2^{mm} longis. Calycis tubus 2^{mm} longus, segmenta linearia 7^{mm} longa. Corollae tubus totus 3^{cm} longus, pars cylindrica 1.5^{mm}-diametralis, lobi obovato-orbiculares 8^{mm} longi emarginati. Stamina inclusa, filamentis per paria leviter connatis, majoribus 8^{mm} longis minores dimidio superantibus, antheris 2.5^{mm} longis. Discus 0.5^{mm} altus. Ovarium glabrum oblongo-lineare 5^{mm} longum 12-ovulatum triente contractum et vacuum, stylo puberulo 31^{mm} longo. Capsula matura ignota.

Ad praedium *Concepción* vocatum, Depart. Escuintla, Guatemala, alt. 400^{m}, Apr. 1890, *John Donnell Smith*, n. 2115 ex Pl. Guat. etc. quas ed. Donn. Sm. (Sub *Ruellia*. sp. olim distributa.)—Ad ripas fluminis Ocosito prope pagum *Caballo Blanco*, Depart. Retalhuleo, Guatemala, alt. 80^{m}, Apr. 1892, *John Donnell Smith*, n. 2692 ex Pl. Guat. etc. quas ed. Donn. Sm.

Pseuderanthemum verapazense Donn. Sm.—Folia lanceolato-oblonga in acumen obtusum angustata basi acuta praeter costam subtus viscidulo-puberulam glabra. Spica terminalis simplex vel ramis binis aucta bracteis foliaceis lineari-oblongis fulta, bracteolis triangulari-linearibus calyce bis brevioribus, floribus singulis subsessilibus.

Caules e rhizomate longe repente hinc illinc erecti subsimplices 10–25^{cm} longi teretes bifariam viscido-pubescentes. Folia lineolata 5–7^{cm} longa 18–20^{mm} lata, petiolis pubescentibus 3–4^{mm} longis. Spica puberula 3–7^{cm} longa, ramis 2–3^{cm} longis, bracteis 12^{mm} longis 3^{mm} latis, bracteolis 1.5^{mm} longis et floribus puberulis. Pedicellus 0.5^{mm} longus. Calycis segmenta linearia acute attenuata 3–3.5^{mm} longa. Corollae laete azureae (cl. repertor in schedula), tubus 16^{mm} longus triente superiore paulo ampliatus ceterum vix 0.5^{mm}-diametralis, lobi elliptici 7.5^{mm} longi 4^{mm} lati. Stamina ad 6^{mm} infra fauces affixa, filamentis 2^{mm} longis, antherarum loculis oblongis 1.5^{mm} longis aequalibus basi acutis, staminodiis 1^{mm} longis. Ovarium puberulum 4-ovulatum, stylo 16^{mm} longo bilobo. Capsula ignota.—*P. hispidulo* Radlk. proximum.

In silvis montanis ad Yaxcabnal, Cubilquitz, Depart. Alta Verapaz, Guatemala, alt. 320^{m}, Mart. 1902, *H. von Tuerckheim*, n. 8258 ex Pl. Guat. etc. quas ed. Donn. Sm.

Dicliptera (§SPHENOSTEGIA Nees) **podocephala** Donn. Sm.—Folia breviter petiolata ovato-lanceolata subsensim acuminata basi

angustata. Pedunculi axillares 1–3-ni elongati monocephali, captulis lineari-bracteatis, involucris 3–8 uni- vel bi-floris, phyllis involucralibus apice rotundato cuspidatis, bracteolis 4 calyce bis longioribus. Corolla paulo exserta.

Fruticulosa 1–1.5-metralis diffusa, ramis obtuse sex-angularibus uti petioli pedunculi involucra lineolatis et puberulis. Folia membranacea glabra cystolithis striolata 8.5–12.5^{cm} longa 3.5–4.5^{cm} lata, petiolis 13–17^{mm} longis. Capitula omnia pedunculata, pedunculis 1–9.5^{cm} longis in axillis superioribus singulis, in inferioribus binis vel ternis inaequalibus. Bracteae capitula semicircularia fulcientes binae 4–7^{mm} longae, involucris plerumque 5, exterioribus decrescentibus, phyllis obovatis basi cuneatis pergameneis nervosis reticulatis per paria inaequimagnis, majore 11–14^{mm} longo 8–9^{mm} lato in statu sicco colorato, altero quarta parte minore viridi, bracteolis lanceolato-linearibus 8^{mm} longis, flore altero abortivo vel deficiente. Calycis laciniae setaceae cum bracteolis puberulae. Corolla 14^{mm} longa, limbo pubescente. Antherarum loculi inaequialte affixi. Stylus 12^{mm} longus. Capsula pubescens ovoidea 5^{mm} longa apiculata disperma, seminibus puberulis 2.5^{mm}-diametralibus.

In pratis humedis ad praedium *Atirro* vocatum, Prov. Cartago, Costa Rica, alt. 600^{m}, Apr. 1896, *John Donnell Smith*, n. 6685 ex Pl. Guat. etc. quas ed. Donn. Sm.

Justicia (§ DIANTHERA Lindau) **Tuerkheimiana** Donn. Sm.—Pilosa eglandulosa. Folia oblongo-ovata apice obtusiuscula basi cuneata sparse paleaceo-pilosa. Spica terminalis pedunculata folia superiora subaequans, bracteis laxe imbricatis lanceolato-ellipticis. Calycis segmenta acuta. Corollae tubus tenuissimus.

Herba procumbens diffusa ad genicula radicans 23–30^{cm} longa, ramis obtuse tetragonis et petiolis et pedunculo patenter pilosis, glandulis obsoletis. Folia ejusdem paris parum inaequalia 23–38^{mm} longa 12–19^{mm} lata lineolata, nervis lateralibus utrinsecus 4–5, petiolis 4–10^{mm} longis. Pedunculus circiter 11^{mm} longus. Spica 24–27^{mm} longa, bracteis 5–9^{mm} longis 2–3^{mm} latis utrinque acutis deorsum autem longius angustatis sparse pilosis ciliatis, bracteolis lineari-lanceolatis 3^{mm} longis. Calycis segmenta lineari 4^{mm} longa, postico filiformi 2^{mm} longo. Corollae tubus 4^{mm} longus vix 0.5^{mm}-diametralis, labia extus puberula 2^{mm} longa. Filamenta 1.5^{mm} longa, antherarum loculis ovalibus inaequalibus breviter disjunctis. Ovarium 1^{mm} altum 4-ovulatum, stylo 5^{mm} longo. Capsula ignota.—*J. Schenckianae* Lindau arcte affinis.

Ad fissuras saxorum, Cubilquitz, Depart. Alta Verapaz, Guatemala, alt. 350^{m}, Jun. 1903, *H. von Tuerckheim*, n. 8726 ex Pl. Guat. etc. quas ed. Donn. Sm.

BALTIMORE, MD.

BRIEFER ARTICLES

NEW NORMAL APPLIANCES FOR USE IN PLANT PHYSIOLOGY. V[1]

(WITH TWO FIGURES)

In the preceding articles I have described some ten new pieces of apparatus designed for educational work in plant physiology; accounts of three more are given below, while others are to follow. They are called normal appliances because they are intended to represent the optimum resultant, the harmonic optimum, as it were, between accuracy of results and convenience of use, while at the same time they can always be bought from the stock of a supply company. As in the case of the other pieces these are to be manufactured and sold by the Bausch & Lomb Optical Company of Rochester, N. Y.

X. Space markers

For some purposes, especially in the study of growth, it is necessary to mark off a structure into regular divisions, either areas as in the case of young leaves, or lengths as in roots, stems, or petioles. It is not difficult to improvise appliances for accomplishing these ends, but as yet no tools are available for effecting them quickly, accurately, and conveniently, while at the same time always ready for use. This need, I think, the two little instruments here described will supply.

First, for marking lengthwise, the instrument is a wheel, the rim of which is a rubber stamp having raised cross-lines 2^{mm} apart. It revolves freely but evenly on an axle held in the end of a handle, and when suitably inked by the method described below, it may be run rapidly over long structures such as roots, marking them with narrow black cross-lines equally spaced, precisely as shown at the bottom of *fig. 1*.

Second, for marking areas, the instrument is a disc, likewise a rubber stamp, having raised lines in the form of squares 2^{mm} on a side. It works by means of a scissors-frame against a cushion disc covered with soft felt and provided with a radial slot to admit the petiole of a peltate leaf. When the marking disc is inked and pressed firmly against a leaf held on the cushion disc, it marks a network of even fine black lines like the sample shown in *fig. 1*. The marking disc is hinged to its supporting arm in a

[1] Continued from BOT. GAZETTE **43**:279. April 1907.

way to permit the disc to settle evenly upon the leaf surface no matter what the thickness of the leaf.

Both instruments may be inked from an ordinary rubber-stamp pad, and the black record kind gives good results. Better, however, is a simple

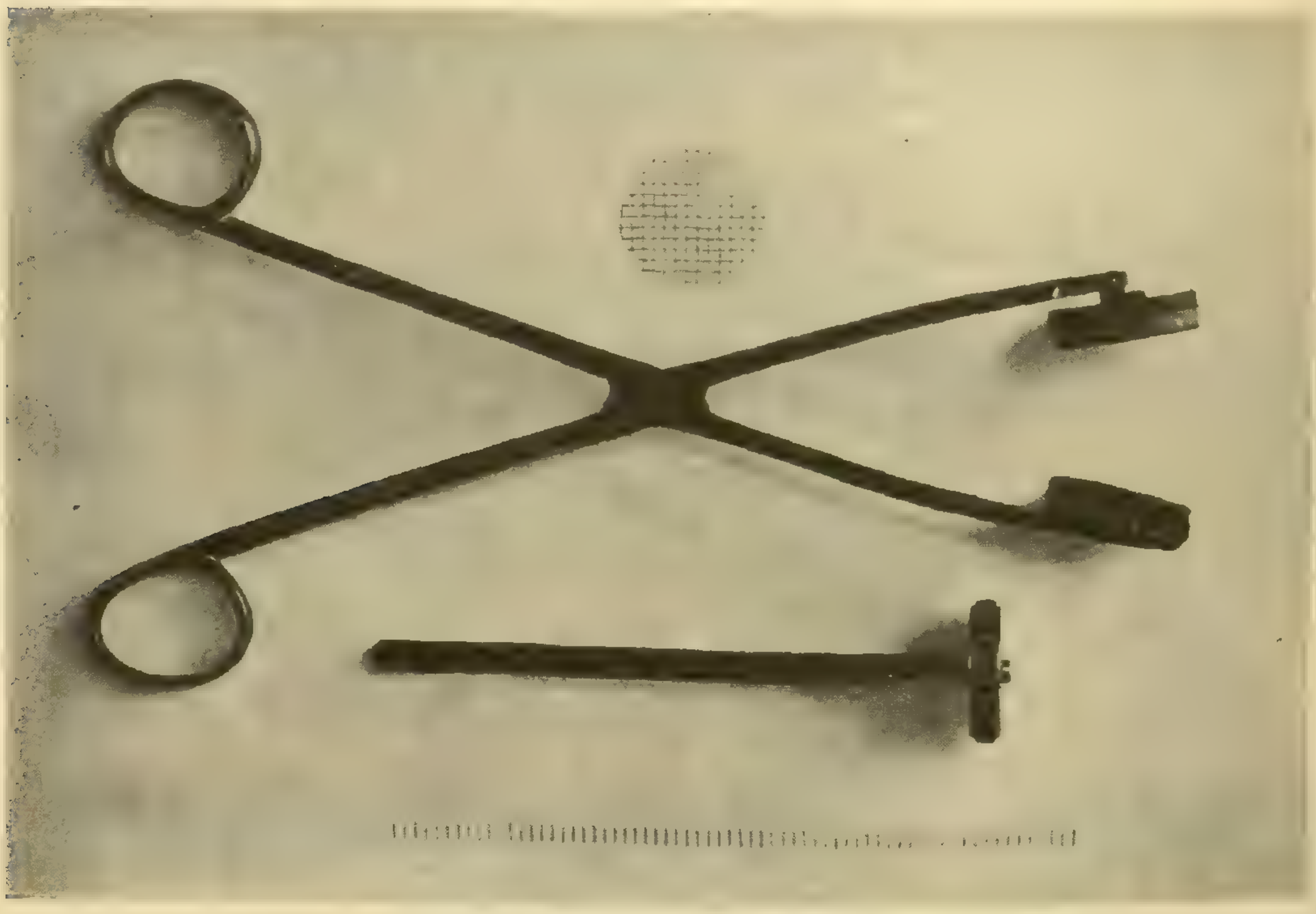

FIG. 1.—Space markers. $\times\frac{1}{2}$.

pad made from one fold of thin close cotton cloth attached by thread to an ordinary glass slide, and inked when needed by a mixture of three parts Higgins' waterproof India ink and one part glycerin.

XI. Demonstration auxograph

Among the most important of the topics which all teachers desire to demonstrate in general botanical courses is growth, and this can be shown to complete satisfaction only through use of a recording instrument. Many recording auxanometers, or auxographs, have been described, but as yet no practical instrument for educational purposes is obtainable by purchase. The essentials of a good demonstration instrument, aside from easy applicability to its work and durability, are reasonable accuracy, ready portability, visibility of record from some distance, and clear exhibition of its mechanism and principle. These ends, I believe, are well met in the instrument here described and illustrated (*fig. 2*).

It consists essentially of four parts: support-stand, recording cylinder, magnifying wheel, and plant-support. The support-stand is of rigid

though thin steel, and is provided with convenient handles, leveling screws, and suitable holes for the two upright rods. The recording cylinder,

FIG. 2.—Demonstration auxograph. ×½.

supported and guided at the top by a screw pivot, turns once an hour, and is of such circumference that each millimeter of a millimeter record paper represents one minute. It is carried by a clock which is supported at such

a height that it may be wound and regulated from beneath without disturbing the record. The magnifying wheel, really four concentric wheels combined, allows three degrees of magnification, two, four, and eight times the actual growth. It is made of aluminum, moves on a very sensitive axle, has suitable openings for attachment of threads, and is provided with a clamp for holding it immovable while adjustment of threads and the like is being made. The tip of the plant is brought into action with the wheel by means of a fine thread in the usual way; but in order that this thread may be kept as short as possible, a plant support, adjustable for height, is provided on a separate rod, thus permitting the tip of the plant to be kept close to the magnifying wheel, though, of course, care must be taken to prevent the danger of shading, and hence of phototropic bendings. This adjustable support, however, has another very important use which will be mentioned below. The thread from the large wheel passes over a pulley to the pen carrier, which slides on a fine guide wire and has sufficient weight to turn the wheels in proportion as the growth of the plant permits the small wheel to turn. The pen is of glass, drawn to a capillary point and bent so as to rest at right angles to the paper. It is filled with chronograph ink, and, as the plant grows and the cylinder turns, it traces a fine spiral line down the cylinder, crossing any given vertical line once an hour. When this pen has reached the bottom of the cylinder, one has only to put on a new cylinder or record paper, turn the large wheel backward until the pen is drawn to its top, close the clamp to hold the wheel immovable, lower the plant support until the thread from the plant becomes again taut, loosen the clamp to allow the tensions to adjust themselves, and then the record is resumed; and this procedure can be repeated until the end of the experiment without any need for ever touching the threads. This is the other advantage, above mentioned, of the adjustable plant-support. One should never draw up the pen by lowering the plant support, as there is a constant temptation to do, since this brings an unnatural strain upon the plant tip. All parts of the instrument, even to the arms carrying magnifying wheel and pulley, are adjustable, so the instrument may be made to work smoothly under any conditions. While designed primarily for making records of growth, it can be used for any measurements involving movement, e. g., the rise of water in a tube.

The weak point of all auxographs lies in the threads, which will alter length hygroscopically and thus introduce error into the record, despite any known treatment with wax, oil, rubber, etc. These alterations may be minimized by treating the threads with wax, and by keeping them as short as possible, for which reason they should be made only long enough to

allow the turning of the wheels, with no extra turns around the latter. The error, of course, is greatest from the thread attached to the plant, since its alterations of length are magnified in the record. Not only should this thread be kept short, but I think a glass filament could advantageously be substituted for all of its length except the loop at the plant and the partial turn around the wheel. The results of these errors can also be relatively minimized by using plants of the most rapid and vigorous growth, such as the flower-stalks of bulbous plants.—W. F. GANONG, *Smith College, Northampton, Mass.*

CURRENT LITERATURE

BOOK REVIEWS

Anisophylly

A monograph on this subject has been prepared by FIGDOR,[1] *privat-docent* in plant physiology in the University of Vienna. It is inspired by WIESNER, the distinguished physiologist of that university, and is dedicated to him. Naturally enough it is dominated by his views, and is interspersed with quotations from his writings. FIGDOR has gathered together what is at present known regarding anisophylly and has presented (critically, he says) the subject from both the morphological and the physiological point of view. In space, at least, the former predominates; and it must be confessed that the physiology is too obscure yet to be very satisfactory.

In the first section (36 pp.) the author defines the term, states the history of the phenomenon, and describes, as various sorts, incomplete, exorbitant, complete, lateral, habital, secondary, and false anisophylly. The second section (67 pp.) describes all the cases of anisophylly observed, in the systematic order from lycopods to composites, including cases of anisocotyly. The third section (55 pp.) discusses briefly the branching and symmetry of anisophyllous plants. The fourth and most interesting section (55 pp.) treats the causes of anisophylly—light, gravity, precipitation, nutrition, transpiration, exotrophy, and correlations. This fourth section would have been much shorter had FIGDOR discussed only causation; he intends to enumerate experimental work on all recorded cases, including new observations of his own. He has overlooked, however, the beautiful demonstration of DORETY[2] that gravity is the cause of anisocotyly in Ceratozomia; nor among the anisophyllous gymnosperms does he mention this and other cases in cycads.

The general conclusion is that anisophylly, which is very much more general than is commonly thought, cannot be said to be due in nature directly to the specific single causes to which experiments often point; but rather to the complex of factors, external and internal, which act on the primordia of the leaves. Primarily, therefore, the position of these is important, and that is conditioned by the orientation of the axes which bear them, with all its complex causation. But anisophylly may be in part phylogenetic, as well as ontogenetic; and here we

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[1] FIGDOR, W., Die Erscheinung der Anisophyllie: eine morphologisch-physiologische Studie. 8vo. pp. viii + 175. *figs.* 23. *pls.* 7. Franz Deuticke: Leipzig und Wien. 1909.

[2] DORETY, HELEN A., The seedling of Ceratozamia. BOT. GAZETTE **46**:305. 1908.

meet factors of whose value we are ignorant. That ignorance needs emphasis. To say—"anisophylly is to be looked upon as a special case of anisomorphy, by which we understand, with WIESNER who proposed this term, that fundamental property of living substance in consequence of which the different organs (in our case the foliage leaves) have the power, each according to its position toward the horizontal or toward the parent axis, to assume different typical forms"—is merely to cloak ignorance with pedantry.—C. R. B.

Desert vegetation

A very fitting celebration of the fifth anniversary of the establishment of the Desert Laboratory was the publication of a treatise on North American deserts by Dr. MACDOUGAL, the director of the department of botanical research of the Carnegie Institution.[3] Had there been any doubt concerning the wisdom of establishing the Desert Laboratory, it must have been long ago dispelled by the number of valuable contributions emanating from Tucson. The contribution here considered includes some of the matter that made up the body of the first report on our desert region by COVILLE and MACDOUGAL in 1903 (Publication 6), but the great amount of new material, based on subsequent explorations and on the investigations at Tucson, made imperative the publication of a general treatise of this sort. An account is given first of the earlier investigations of the institution and the development of the department of botanical research, especial attention being directed to problems of long continuance, such as the study of the revegetation of the Salton Basin and experiments on acclimatization. Nearly half of the work is devoted to a general account of the various desert regions of North America, including the various Mexican deserts, the northern sage-brush deserts, the Mohave desert and Death Valley, the Sonoran and Colorado deserts. Then follows a sketch of the geological features of the region about Tucson, by Professor W. P. BLAKE, territorial geologist of Arizona; herein is contained an account of the soils, including the *caliche*, an interesting calcareous formation arising through deposition from waters percolating upward. An interesting sketch is given of the seasonal changes in the aspect of the vegetation about Tucson. The early winter rains stimulate the development of a number of winter perennials and annuals. In the spring and early summer the aspect is controlled by more xerophytic spinose and succulent forms, notably the cacti. The humid mid-summer, like the winter, is characterized by a number of forms stimulated to development by the greater moisture. The treatise closes with a consideration of temperatures of plants in the desert (it being suggested that the great difference between air and soil temperatures is likely to be of significance), the water relations of desert plants, soil relations of desert plants, conditions contributory to deserts, and some general remarks on the formation and extent of deserts and the influence of the desert on life. This treatise will be a *sine qua non* for all ecological workers, since it brings together what is known concerning our deserts, taking

[3] MACDOUGAL, D. T., Botanical features of North American deserts. Carnegie Institution of Washington, Publication 99. 1908.

account of collateral information as well as the researches at the Desert Laboratory.—HENRY C. COWLES.

Animal galls

A prodigious amount of work is represented by the two ponderous volumes of HOUARD, devoted to the galls, produced by animals, which have been found upon European plants, including those of the Mediterranean basin.[4] Few botanists, we imagine, are aware of the extent of what now really ranks as a special branch of biological science, cecidology, which has its own journal, *Marcellia*, and is awakening the interest of both botanists and entomologists.

In this monumental work HOUARD describes 6239 zoocecidia, produced by 1466 species of animals, on 2299 species of plants. Of the animal gall-producers the most important are the Insecta, of the families Curculionidae (Coleoptera), Cynipidae (Hymenoptera), Cecidomyidae and Muscidae (Diptera), Aphididae (Hemiptera), and the Arachnida, of the family Eriophyidae. The Nematodes furnish 16 cecidogena, of the family Anguillulidae; while even Copepoda and Rotifera are represented by one species each. Of the plants 68 are cryptogams, 35 gymnosperms, 173 monocotyls, and 2053 dicotyls.

A large number of the galls are illustrated by original figures and some copies, both external and sectional views being given when necessary to show structure. The part of the plant deformed is indicated; the gall is described tersely, with compact and inconspicuous bibliographical notations; the specific name of the animal responsible for the deformity is given when known, otherwise the best data available; and finally an indication of the geographical distribution is added. There is a full bibliography, arranged alphabetically by authors; an index to the animals named, preceded by a tabular view of the genera, classified by families and orders; and an index of the plants by genera and species.

It is not often one sees a scientific work involving such multifarious detail planned so carefully and carried out so consistently and successfully. Herein the publishers doubtless deserve praise for active cooperation. It would be difficult to find a flaw in either plan or execution.

Since no extensive studies on the cecidia of this country have been made, these volumes, the most thorough, comprehensive, and accurate that have yet appeared in any country, will doubtless serve for many years in the preliminary work that needs to be done on our own galls. They must certainly be most useful, and it is to be hoped that with such a guide, more of our younger biologists will take up the study with vigor.—C. R. B.

A Darwin memorial volume

Among the numerous publications in commemoration of the centenary of the birth of CHARLES DARWIN and of the fiftieth anniversary of the publication of

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[4] HOUARD, C., Les zoocécidies des plantes d'Europe et du bassin de la Méditerranée. 2 vols. 8vo. pp. 1248. *figs. 1365. pl. 2. portraits 4.* Paris: A. Hermann & Fils. 1909. 45 *fr.*

the *Origin of species*, no one seems more appropriate and satisfactory than the volume issued by the Cambridge Philosophical Society and the Syndics of the University Press.[5] It consists of twenty-eight essays written by those who are most competent to present the various appropriate topics. The result is to illustrate the far-reaching influence of DARWIN'S work and also the present attitude of investigators toward Darwinism. Some of the essayists have restricted themselves to DARWIN'S own work; while others have outlined the progress of more recent research, which has been the direct outcome of his work. Two photographs of DARWIN are reproduced, one made in 1854, and the other in 1880; while a reproduced etching gives a most interesting view of the study at Down. To review such a collection of essays briefly is impossible, but the subjects and the authors will indicate the general contents to those interested in evolutionary doctrine. Ten of the twenty-eight essays are of interest to botanists.

The series most appropriately begins with an introductory letter by Sir JOSEPH D. HOOKER, for forty years the intimate friend and correspondent of DARWIN. The ten essays of botanical interest are as follows: "Darwin's predecessors," by J. ARTHUR THOMSON (15 pp.); "The selection theory," by AUGUST WEISMANN (48 pp.); "Variation," by HUGO DE VRIES (19 pp.); "Heredity and variation in modern lights," by W. BATESON (17 pp.); "The minute structure of cells in relation to heredity," by EDUARD STRASBURGER (10 pp.); "The palaeontological record. II. Plants," by D. H. SCOTT (23 pp.); "The influence of environment on the forms of plants," by GEORG KLEBS (24 pp.); "Geographical distribution of plants," by W. T. THISELTON-DYER (21 pp.); "Darwin's work on the movement of plants," by FRANCIS DARWIN (16 pp.); "The biology of flowers," by K. GOEBEL (23 pp.)

All of these essays address themselves primarily to the intelligent public rather than to investigators, and therefore they are in a sense popular statements and not contributions to knowledge. In spite of this, they are very interesting to investigators, for personal and recent points of view are in evidence throughout, and the whole group of related topics is brought together in clear and compact form.—J. M. C.

MINOR NOTICES

Recent publications from the National Herbarium.—A. S. HITCHCOCK (Contr. U. S. Nat. Herb. 12:183–258. 1909) has issued a "Catalogue of the grasses of Cuba." The work is based primarily on material in the herbarium of the Experiment Station at Santiago de las Vegas, Cuba. Sixty-six genera are recorded and to these are referred 225 species of which 10 are indicated as new; one new genus (*Reimarochloa*) is proposed. More than one-half (36) of the genera here listed are represented by single species. The author gives carefully prepared keys leading to the genus and species and also cites numerous exsiccatae, thus greatly

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[5] Darwin and modern science. Essays edited by A. C. SEWARD. 8vo. pp. xvii + 590. Cambridge: The University Press. 1909. $5.00.

enhancing the practical value of the publication.—J. N. ROSE (*ibid.* 259–302) has published the sixth paper in his "Studies of Mexican and Central American plants." About 75 species are described as new, and several transfers have been made. Three new genera (*Pelozia*, *Pseudolopezia*, and *Jehlia*) of the Onagraceae are briefly characterized. The text is supplemented by numerous illustrations.—P. C. STANDLEY (*ibid.* 303–389. *pls. 28–43*) publishes an interesting systematic treatment of the Allioniaceae, dealing chiefly with those of the United States. The author recognizes 16 genera, describes about 50 species and some 20 so-called sub-species as new to science; three of the genera enumerated are new, namely *Anulocaulis*, *Commicarpus*, and *Hesperonia*. Through an apparent oversight the genus Commicarpus either has been omitted from the key to the genera or confused with Senkenbergia.—N. L. BRITTON and J. N. ROSE (*ibid.* 391, 392. *pls. 44, 45*) propose a new genus (*Thompsonella*) of the Crassulaceae; the genus is represented by two Mexican species. The type of the genus is *Echeveria minutiflora* Rose.—J. N. ROSE (*ibid.* 393–409. *pls. 46–59*) describes 10 new species of flowering plants chiefly from Mexico and the southwest, including also a new genus (*Conzattia*) of the Leguminosae, and makes critical notes on species previously published.—N. L. BRITTON and J. N. ROSE (*ibid.* 413–437. *pls. 61–76*), in an article entitled "The genus Cereus and its allies in North America," have recorded 24 genera, of which 15 are designated as new. Of the 131 species enumerated 12 are described as new, 77 form new combinations, and 19 are of doubtful generic relationship. The new genera proposed are as follows: *Acanthocereus*, *Bergerocactus*, *Heliocereus*, *Hylocereus*, *Lemaireocereus*, *Leptocereus*, *Lophocereus*, *Nyctocereus*, *Pachycereus*, *Peniocereus*, *Rathbunia*, *Selenicereus*, *Weberocereus*, *Werckleocereus*, and *Wilcoxia*.—J. N. ROSE (*ibid.* 439, 440. *pls. 77–81*) describes and illustrates 5 new species of Crassulaceae from Mexico.—J. M. COULTER and J. N. ROSE (*ibid.* 441–451. *pls. 82, 83*) have issued a "Supplement to the monograph of the North American Umbelliferae" in which the authors include descriptions of 6 new species; two new genera are also proposed, namely, *Ligusticella* and *Orumbella*.—W. R. MAXON (*ibid.* 411. *pl. 60*) describes and illustrates a new species of Asplenium from China; and (*ibid.* 13:1–43. *pls. 1–9.* 1909) in continuation of a series of articles begun in an earlier volume of this journal has published results of further studies of tropical American ferns. In this paper, the second of the series, the author describes 16 species of ferns and 2 species of Lycopodium from Mexico and Central America, and also presents a "Revision of the West Indian species of Polystichum" in which 19 species are recognized, 4 being hitherto undescribed.—J. M. GREENMAN.

A garden book.—A lover of flowers will find pleasure and inspiration in a charming book entitled *A little Maryland garden* by HELEN ASHE HAYS.[6] She writes in a most interesting and pleasantly intimate way of her experiences in starting a flower garden, of her successes and failures, and of the great satisfaction

[6] HAYS, HELEN ASHE, A little Maryland garden. 12 mo. pp.——. *pls. 8.* New York: G. P. Putnam's Sons. 1909. $1.75.

one derives from a garden which is *klein, aber mein*. The book is beautifully illustrated with eight halftones in color by ZULMA DE L. STEELE, and would be a pleasing gift book, as well as an excellent reference book for an amateur who may feel, with the author, that "at least it is better to have tried and failed, than not even to have made the attempt." The author, however, was evidently successful and shows an intimate knowledge of garden life. The nature sketches are pleasing, and the whole book is written in a very happy vein, to which its attractive form is appropriate.—MARY H. FROST.

Hymenomycetes of the Chicago region.—The Natural History Survey of the Chicago Academy of Sciences has begun the publication of a descriptive catalogue of the higher fungi of the Chicago area. The first part, containing the Hymenomycetes by MOFFATT, has just appeared.[7] It is well printed and the plates are halftones from excellent photographs. The keys to genera and species should make determination comparatively simple, but the key to genera would be far more convenient if the page numbers were inserted. The "Chicago area" means Cook and Dupage counties, with portions of Will County, Ill., and Lake County, Ind., including about 1800 square miles. From this area 371 species of Hymenomycetes are reported, representing 79 genera, the distribution by families being as follows: Agaricaceae, 46 gen., 211 spp.; Polyporaceae, 15 gen., 78 spp.; Hydnaceae, 5 gen., 25 spp.; Thelephoraceae, 8 gen., 41 spp.; Clavariaceae, 2 gen., 12 spp.; Tremellaceae, 3 gen., 4 spp.—J. M. C.

Indian woods and their uses.—The Imperial Forest Research Institute of India has begun the publication of a series of memoirs, the first number of which deals with Indian woods and their uses.[8] It is a bulky quarto volume of nearly 500 pages, dealing wih 554 species. This is only a fraction of the total number ot Indian woody species, which is said to be about 5000 and rather more than half of them trees. The first part contains a list of the purposes for which woods are employed and the woods used for each, while in the second part these woods are described. There is an index to English and trade names (9 pp.), and also a surprisingly extensive one (202 pp.) to vernacular names.—J. M. C.

The flora of central and southern Congo.—Another fascicle[9] of this important taxonomic work has been issued recently under the able editorship of Professor ÉM. DE WILDEMAN. The present fascicle contains a list of Mycetes prepared by the late Professor P. HENNINGS, also a list of fungi by H. and P. SYDOW; the Pteridophyta have been elaborated by Dr. H. CHRIST and the Embryophyta by Dr. DE WILDEMAN. Nearly one hundred new species and several varieties are

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[7] MOFFATT, W. S., The higher fungi of the Chicago region. Part I. The Hymenomycetes. Chicago Acad. Sci. Nat. Hist. Surv. Bull. **7**: 1–156. *pls. 1–24.* 1909.

[8] TROUP, R. S., Indian woods and their uses. Indian Forest Memoirs **1**: No. 1. 4to. pp. 273+ccxvii. 1909.

[9] DE WILDEMAN, ÉM., Flore du Bas- et du Moyen-Congo. Ann. Mus. Congo. Botanique, Sér. V. Tome iii. fasc. 1. pp. 147. *pls.* 27. Brussels. 1909.

here published, and the text is supplemented by twenty-seven full-page illustrations.—J. M. GREENMAN.

Handbook of deciduous trees.—The ninth part[10] of SCHNEIDER'S *Handbook* (the fourth section of the second volume) has followed the preceding one[11] with great promptness. As already noted, it presents descriptions of the species of angiospermous trees, native or under cultivation in central Europe, and is illustrated freely. The present part begins with Tilia and ends with Rhododendron. —J. M. C.

NOTES FOR STUDENTS

Morphology of Tumboa.—Three years ago PEARSON published[12] the results of his investigation of Tumboa (Welwitschia) from material obtained in one day's collecting. A second expedition to Damaraland was made possible and material was collected during January and February of 1907, the results of the investigation of which are now published.[13] The additional stages thus secured have put our knowledge of this most interesting plant upon a fairly substantial basis, and PEARSON is to be thanked for his persistent enthusiasm in securing this difficult material. An outline of what seem to be the most significant new results is as follows:

The staminate and ovulate strobili are often produced in great profusion and their occurrence below the single pair of leaves is frequent. Pollination is mainly effected by a hemipterous insect (Odontopus), the pollen being received by a nectar drop on the top of the projecting micropylar tube. The pollen grains frequently germinate in the micropyle at some distance from the tip of the nucellus, the tube growing down through the fluid which fills the micropyle at the time of pollination. The generative cell passes into the tube, where its nucleus divides, the binucleate cell either remaining undivided or forming two male cells. The tube nucleus begins to break down before fertilization and eventually disappears.

The most critical and puzzling structure of Tumboa, however, is the embryo sac. Megaspores and embryo sacs are often present in the pith region of the axis of the ovulate strobilus, so that the cauline origin of the ovule is clear. A single megaspore mother cell is organized and a single megaspore functions. The female gametophyte begins with free nuclear division and no vacuolation, and successive simultaneous divisions occur until there are approximately 1024 free and crowded nuclei. Elongation of the sac then occurs, chiefly in its micropylar

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[10] SCHNEIDER, C. K., Illustriertes Handbuch der Laubholzkunde. Neunte Lieferung (vierte Lieferung des zweiten Bandes). Imp. 8vo. pp. 367–496. *figs. 249–328.* Jena: Gustav Fischer. 1909. *M* 4.

[11] BOT. GAZETTE **47**:415. 1909.

[12] PEARSON, H. H. W., Some observations on *Welwitschia mirabilis* Hooker. Phil. Trans. Roy. Soc. London B **198**:265–304. *pls. 18–22.* 1906. Review in BOT. GAZETTE **42**:67. 1906.

[13] ———, Further observations on Welwitschia. Phil. Trans. Roy. Soc. London B **200**:331–402. *pls. 22–30.* 1909.

fourth, so that the outer nuclei are more widely separated than the rest. These more scattered nuclei are sexually functional, while the more crowded ones in the inner three-fourths of the sac give rise to the endosperm. Incomplete wall-formation occurs, dividing the sac into irregular and multinucleate compartments, those of the upper fourth usually containing not more than six nuclei, while those of the lower three-fourths contain twelve or more nuclei. The outer multinucleate cells develop tubular prolongations (prothallial tubes) into the nucellus, into which the nuclei and most of the cytoplasm pass. Occasionally these sexual nuclei fuse within the prothallial tube. In the multinucleate cells of the inner three-fourths of the sac the nuclei seldom divide, but all fuse, forming uninucleate cells. This endosperm, consisting of uninucleate cells whose nuclei are formed by the fusion of what the author regards as potential gametes, he calls a *trophophyte*, to distinugish it from both gametophyte and sporophyte, and says that it "differs fundamentally from the prothallus of the lower gymnosperms," a statement which will have to be amended in a way that will make the proposed name seem unnecessary.

When connection is established between the tip of a pollen tube and of a prothallial tube, "the leading female nucleus enters the generative cell within which fertilization occurs," which is certainly a remarkable performance.

In embryo-formation, the fertilized egg elongates to form a proembryonal tube, toward the tip of which the nucleus moves and divides, when a tip cell is cut off by an ingrowing wall, just as in Gnetum. The tubular cell of the proembryo continues to elongate, while the tip cell develops the embryo, which consists of about sixty cells when its tip reaches the endosperm.

The author enters into a somewhat extended discussion of the general bearings of the facts he has uncovered, a discussion which will be considered in another connection.—J. M. C.

Mechanism of photeolic movements.—LEPESCHKIN, whose investigations of turgor mechanisms have been already extensive and important, has added a study of the mechanism concerned in the so-called sleep movements of leaves, which he designates as photonastic.[14] (It seems to the reviewer much better to reserve the terms compounded of *-nastic* for the irreversible movements due to growth. As an equivalent of the cumbrous *Variationsbewegungen* eolic movements may be suggested. Long since[15] I proposed for the sleep movements the term photeolic movements, avoiding thus the false implications of sleep, nyctitropic, and photonastic.) Without referring to the divergent views of various authors on which his

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[14] LEPESCHKIN, W. W., Zur Kenntnis des Mechanismus der photonastischen Variationsbewegungen und der Einwirkung des Beleuchtungswechsels auf die Plasmamembran. Beih. Bot. Centralbl. **24**:308–356. 1909. Preliminary paper: Zur Kenntnis des Variationsbewegungen. Ber. Deutsch. Bot. Gesells. **26a**:724–735. 1908.

[15] HEALD, F. D., Contribution to the comparative histology of pulvini and the resulting photeolic movements. BOT. GAZETTE **19**:480. 1894.

conclusions bear, an attempt is made here to state clearly and tersely LEPESCHKIN'S conception of the various processes concerned in the movements by motor organs.

A change in illumination induces a change in the permeability of the plasma membranes for solutes; this results in an alteration of the turgor pressure, which of course alters the volume of the opposed halves of the motor organ, and alters it in the same fashion, though not at the same rate or to the same extent. Darkening reduces permeability, and consequently increases the turgor; lighting has the opposite effect. The result of the inequalities of these changes in turgor is a curvature of the pulvinus. After this has appeared, diffusion of the solutes begins toward the convex side, where the concentration is now lowest in consequence of the absorption of water in this half and its expulsion on the concave side; this leads to the restoration of the normal concentration of sap and a resultant heightening of turgor on the convex side, with a corresponding lowering of it on the other, thus intensifying the curvature. Alteration of permeability by changes of illumination is not peculiar to motor organs, but occurs also in epidermal cells of Tradescantia and in Spirogyra, where it is proportionally as great, but cannot have the same consequences.[16] Of the two movements ordinarily induced by change in illumination, the rise or fall of the leaf and the reverse, only the primary movement is produced as described; the reverse movement is rather of the nature of an after-effect of the primary curvature. Geotropic curvatures of the motor organs are explicable on the same principles. The physiological dorsiventrality of the motor organs is due to the normal direction of gravity. Plants which raise their leaves on darkening, have their photeolic movements intensified by being inverted, while those that drop their leaves have them reversed by inversion.—C. R. B.

Seedling structure of gymnosperms.—The third paper under this title, by HILL and FRAINE,[17] treats of the Ginkgoales and Cycadales, and contains the usual valuable coordination of scattered results. Under the three heads of cotyledons, transition region, and root, the following conclusions are reached:

Cotyledons.—The cotyledons, generally two in number, are hypogeal and are persistently imbedded in the gametophyte; they are frequently unequal and there is a marked tendency to form lobes, and in some cases there is a short basal tube; among Cycadales they are more or less closely fused by their ventral surfaces; stomata are generally present, secretory cells and canals are common, and the vascular bundles are mesarch or exarch in varying degrees; the number of bundles in each cotyledon varies from one to eight, in all cases being greater in the central region than near the base or tip.

Transition region.—The transition phenomena occur rapidly, so that most of the hypocotyl shows root structure; Ginkgo differs from the observed cycads in that it has a rotation of the protoxylem of the cotyledonary traces; in Ginkgo each cotyledonary bundle gives rise to two poles of the root (except in the case of

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[16] See also TRÖNDLE, p. 318.

[17] HILL, T. G., AND FRAINE, E. DE, On the seedling structure of gymnosperms. III. Annals of Botany **23**:433–458. *pl. 30.* 1909.

three cotyledons, when the root is triarch); among cycads the cotyledonary bundles are not of equal value in the production of root structure, and even similarly situated bundles vary in the same species; among the cycads the cotyledonary bundles fuse with the plumular traces and ultimately form a central cylinder of variable structure.

Root.—In Ginkgo there may be an addition of protoxylem elements after the root structure has been organized; in Stangeria the primary root may branch dichotomously; after the initial root structure has been attained, the number of poles may be increased at lower levels.

The paper closes with a very useful table showing the variation in the number of bundles in the base of the cotyledons of the fourteen species discussed, and also the relation of this number to the number of poles in the root structure.—J. M. C.

Adaptation in fossil plants.—In his presidential address[18] at the anniversary meeting (May 24) of the Linnean Society, SCOTT took occasion to outline the evidence for adaptation from fossil plants, which naturally dealt chiefly with the anatomical structures of those ancient vascular plants which he has done so much to elucidate. No one is more competent to state the facts in reference to ancient plants, but the conclusions do not seem to be irresistible. In substance they are as follows: (1) at all known stages in the history of plants there has been a thoroughly efficient degree of adaptation to the conditions existing at each period; (2) the characters of plants always having been as highly adaptive as they are now, natural selection appears to afford the only key to evolution which we possess at present; (3) the paleontological record reveals only a relatively short section of the whole evolution of plants, during which there has not been any very marked advance in organization, except in cases where the conditions have become more complex, as illustrated by the floral adaptations of angiosperms; (4) the simple forms of the present flora are reduced rather than primitive, but such reduction may have set in often at a relatively early stage of evolution, and is therefore consistent with a considerable degree of antiquity in the reduced forms.

These broad statements, quite apart from their application to certain views of adaptation, contain much wholesome truth for those who imagine that the paleontological record, as we know it, represents a continuous succession of "higher and higher" plants, for it is becoming increasingly evident that very highly organized plants existed at the very beginning of our record.—J. M. C.

Morphology of Penaeaceae.—STEPHENS published a preliminary account[19] of his studies among the Penaeaceae which was noticed in this journal.[20] There has now appeared the full account with illustrations,[21] so that the morphological

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[18] SCOTT, D. H., Presidential address before Linn. Soc., 1909. pp. 15.

[19] STEPHENS, E. L., A preliminary note on the embryo sac of certain Penaeaceae. Annals of Botany **22**:329. 1908.

[20] BOT. GAZETTE **45**:365. 1908.

[21] STEPHENS, E. L., The embryo sac and embryo of certain Penaeaceae. Annals of Botany **23**:363–378. *pls. 25, 26.* 1909.

features of this small shrubby group, restricted to the southwestern region of Cape Colony, are fairly before us. Three of the five genera were investigated (Sarcocolla, Penaea, and Brachysiphon), suitable material of the other two (Endonema and Glischrocolla) not being available.

The morphological characters of the three genera examined are the same, so that one account can serve for all. The megaspore mother cell produces four nuclei, usually tetrahedrally arranged, and these migrate to the periphery of the embryo sac, where each gives rise to a group of four nuclei. Three of the nuclei of each group are organized into cells which resemble an egg-apparatus, while the four remaining free nuclei fuse in the center of the sac to form the primary endosperm nucleus, which after fertilization forms a parietal layer of nuclei, walls appearing much later. The embryo has no suspensor, appearing first as a spherical mass of cells, which elongates as the tissues are differentiated and the growing points are organized.

This seems clearly an illustration of the formation of an embryo sac by the cooperation of four megaspores, in this case the product of each megaspore remaining remarkably distinct.—J. M. C.

Embryo sac of Pandanus.—A preliminary note[22] under this title has already been referred to in this journal.[23] The fuller account, with plates, has now been published.[24] Pandanus has long been regarded as a promising primitive monocotyledon, and its investigation is most timely. The general results are as follows: the archesporial cell (presumably solitary) cuts off a parietal cell which gives rise to several layers of cells separating the epidermis from the megaspore mother cell; the mother cell divides transversely into two daughter cells, the inner one of which directly produces the embryo sac, while the outer one divides anticlinally; the first division within the sac (the second reduction division) results in two polar nuclei; the micropylar nucleus divides, and there is no division of the daughter nuclei, nor is there usually any differentiation into egg and synergid; the antipodal nucleus gives rise to twelve nuclei, whether by simultaneous division or not was not determined; in the most advanced stages secured no nuclear fusion was observed, all fourteen nuclei remaining quite separate.

The author still maintains that the embryo sac of Pandanus is a more ancient type than the ordinary eight-nucleate sac of angiosperms, and that it represents a new type, "with its nearest analogue in Peperomia." It remains to investigate the fertilization stages of this interesting embryo sac, to determine whether the fourteen-nucleate condition really is the fertilization stage.—J. M. C.

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[22] CAMPBELL, D. H., The embryo sac of Pandanus. Preliminary note. Annals of Botany **22**:330. 1908.

[23] BOT. GAZETTE **45**:364. 1908.

[24] CAMPBELL, D. H., The embryo sac of Pandanus. Bull. Torr. Bot. Club **36**:205–220. *pls. 16, 17*. 1909.

The new flora of Krakatau.—Under this title CAMPBELL[25] has published an interesting account of a visit to the island of Krakatau, which was "efficiently sterilized" in August 1883, the hot ashes and pumice completely covering the island to an average depth of 30^{m}. The nearest land is an island 19^{km} distant, on which the vegetation was largely destroyed; while Java and Sumatra are 35 and 45^{km} distant. TREUB visited the island in 1886 and 1897, and it was examined again in 1905 and 1906. By 1886, three years after the catastrophe, a considerable number of plants had been established, the ferns predominating in species (11) and individuals, while 9 species of seed plants were sparsely represented. The Cyanophyceae were also found to be of great importance as aids in the establishment of higher vegetation, the blackish slimy films of species of Oscillatoria coating the surface of the ashes. In 1897, while there were almost no trees, most of the island was covered by vegetation, 62 species of vascular plants being recorded (12 pteridophytes, 50 seed plants), and the ferns still predominating in the number of individuals. In the present flora 137 species have been recorded, representing all the principal groups; the ferns are no longer predominant; and the forest vegetation is rapidly encroaching toward the center of the island. There is a remarkable paucity of bryophytes, only two mosses and one Anthoceros having been recorded.—J. M. C.

Mechanism of anthers.—SCHNEIDER, having investigated the tulip carefully, objects[26] to the conclusions of STEINBRINCK that the rupture of anthers is due to the cohesion of the diminishing water with that in the cell walls. In this problem he would distinguish the mechanics of the first rupture, of the first recurvature of the valves, and of their subsequent rolling and unrolling. In Tulipa he finds the first rupture due to the pressure of the growing pollen mass—an explanation already more than a century old. He does not enlighten us as to the remaining processes; possibly they are treated in an earlier paper which we have not seen.[27]

To this paper STEINBRINCK replies at some length,[28] in the usual lively polemic style of our Teutonic friends. Though tulips were out of bloom before. SCHNEIDER'S article came to his attention, his preserved material even furnishes some arguments, which are further supported by an examination of the behavior of a large number of plants of other genera. The only one in which STEINBRINCK is willing to admit that anything but cohesion mechanism plays a part, even in the first opening of the valves, is the rye. In this anther "another strong tissue tension must cooperate, because the broad cleft remains open when one throws the anther

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[25] CAMPBELL, D. H., The new flora of Krakatau. Amer. Nat. **43**:449–460. 1909.

[26] SCHNEIDER, J. M., Zur ersten und zweiten Hauptfrage der Antherenmechanik. Ber. Deutsch. Bot. Gesells. **27**:196–201. 1909.

[27] ———, Der Oeffnungsmechanismus der Tulipanthere. Altstatten, 1908. (Inaugural Dissertation.)

[28] STEINBRINCK, C., Ueber den ersten Oeffnungsvorgang bei Antheren. Ber. Deutsch. Bot. Gesells. **27**:300–312. *figs.* 7. 1909.

at once into water." The first rupture in this case is an explosive one, which scatters some of the pollen, and cannot be due to the cause assigned by SCHNEIDER. —C. R. B.

Mesostrobus, a new genus of Carboniferous lycopods.—WATSON[29] has described the strobilus of a new lycopod from the Lower Coal Measures of Lancashire. It resembles Lepidostrobus, but the sporangium is only attached to the distal half of the horizontal portion of the sporophyll, and the somewhat larger ligule is set in a deep pit. A characteristic point of view is illustrated by the following quotation: "Lepidostrobus would be derived from a cone having sporophylls of this type" (*Bothrodendron mundum*, etc.) "on the adoption of an arboreal habit by the heterosporous lycopods, because radial elongation of the sporangium is the most economical way of increasing the number of spores produced, a necessity for a large tree. If this elongation takes place in the part of the sporophyll between the axis and the insertion of the sporangium, we arrive at a condition much like that of Spencerites, and from that condition we can pass through Mesostrobus to Lepidostrobus."—J. M. C.

Heating of leaves.—It has been known that the evolution of heat may be demonstrated in living plants by using seedlings and flowers, but leaves have not been considered favorable material for this experiment. MOLISCH has now shown[30] that in many cases 3–5^{kg} of leaves, placed in a basket and packed in "excelsior," show a rise in temperature amounting to 20–45° C. within 12–24 hours. The leaves are usually killed thereby, and after a fall a second rise of temperature begins, which may attain a maximum somewhat higher or lower than the first. The first evolution of heat he ascribes to the respiration of the leaves, while the second is due to the rapid development of microorganisms. The experiment is simple and worthy a place in the laboratory practice.—C. R. B.

Osmotic pressure and permeability.—TRÖNDLE records another example of what has been observed by others, namely, change in the permeability of the protoplast according to the conditions of lighting and temperature. His preliminary report[31] concerns the leaves of *Tilia cordata* and *Buxus sempervirens rotundifolia;* in the former both palisade and spongy parenchyma, in the latter only the palisade being investigated. He reports also the high values of 20–26A for the osmotic pressure as determined by plasmolysis. It is to be remembered that plasmolytic studies, such as these, in many of which NaCl was used, are of

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[29] WATSON, D. M. S., On Mesostrobus, a new genus of lycopodiaceous cones from the Lower Coal Measures, with a note on the systematic position of Spencerites. Annals of Botany **23**:379–398. *pl. 27.* 1909.

[30] MOLISCH, H., Ueber hochgradige Selbsterwärmung lebender Laubblätter. Bot. Zeit. **66** :211–233. 1908.

[31] TRÖNDLE, A., Permeabilitätsänderung und osmotischer Druck in den assimilierender Zellen des Laubblättes. Ber. Deutsch. Bot. Gesells. **27**:71–78. 1909.

questionable validity in the light of OSTERHOUT'S researches in this line.[32]—C. R. B.

Anatomy of the ovule of Myrica.—Miss KERSHAW[33] has investigated the ovule of *Myrica Gale*, and has discovered that in all of the morphological features it is an ordinary angiosperm, with its solitary megaspore mother cell, linear tetrad, eight-nucleate embryo sac, and porogamy. The following anatomical features, however, are worthy of mention: the nucellus is not only completely free from the single integument but is also distinctly stalked within it; vascular strands (eight or nine in number) traverse the integument, without branching, almost to the apex of the ovule. These two features of the ovule are usually regarded as primitive, belonging to the ancient gymnosperms rather than to angiosperms.—J. M. C.

Phototropism of roots.—LINSBAUER and VOUK, after overcoming many experimental difficulties, have found[34] that the roots of *Raphanus sativus* and *Sinapis alba*, which have been credited with being only negatively phototropic, react positively or negatively according to the intensity of the light. Roots of the former in moist air turn toward light of about 8 candles, while in water they are much less sensitive, no very certain curvatures being obtained until the light was increased to 400 c.p. Sinapis in water, on the contrary, gave the best positive response at 0.2 c.p., and decided negative curvatures at 0.64 c.p. These results support the MÜLLER-OLTMANNS theory of phototropism.—C. R. B.

Dispersal of seeds by ants.—WEISS[35] has concluded that the gorse (Ulex) and the broom (Sarothamnus) should be included among myrmecochorous plants, along with Chelidonium, Viola, etc. He finds that the seeds have a brightly colored caruncle containing oily food material and resembling in structure and contents the elaiosomes (of SERNANDER) of other myrmecochorous plants; that ants are particularly attracted by the oil-containing caruncle, and can and will carry about the seeds of gorse; and that the rectilinear distribution of gorse bushes along actual or disused paths or roadways is only paralleled by the distribution of such plants as the celandine along ant-runs.—J. M. C.

Anatomy of Gleichenia.—BOODLE and HILEY[36] have investigated the vascular structure of Gleichenia, a genus interesting on account of its protostelic species.

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[32] BOT. GAZETTE **46**: 53–55. 1908.

[33] KERSHAW, EDITH MAY, The structure and development of the ovule of *Myrica Gale*. Annals of Botany **23**: 353–362. *pl. 24*. 1909.

[34] LINSBAUER, K., AND VOUK, V., Zur Kenntnis des Heliotropismus der Wurzeln. Ber. Deutsch. Bot. Gesells. **27**: 151–156. 1909.

[35] WEISS, F. E., The dispersal of the seeds of the gorse and the broom by ants. New Phytol. **8**: 81–89. 1909.

[36] BOODLE, L. A., AND HILEY, W. E., On the vascular structure of some species of Gleichenia. Annals of Botany **23**: 419–432. *pl. 29*. 1909.

G. pectinata was especially studied, whose rhizome BOODLE[37] had discovered to be solenostelic. This has now been confirmed, solenostely with leaf gaps being found. It is concluded that Eugleichenia represents a series of reduction forms from the Mertensia type (represented by *G. flabellata*), and that Mertensia includes the most primitive species as well as the most advanced (*G. pectinata*), in which a solenostelic structure has been derived from a protostelic.—J. M. C.

Ovule of Julianiaceae.—Miss KERSHAW[38] sees in the integumental vascular strands and free nucleus of this recently established Mexican family a suggestion of relationship between Juliania and Juglans, and especially in the association of this structure in both genera with the outgrowth at the base of the ovule known as the obturator. The suggested connection with Anacardiaceae is confirmed by the integumental vascular strands of Mangifera, but in that genus there is no indication of an obturator.—J. M. C.

Chlorophyll in evergreens.—Miss CÄCILIE STEIN reports[39] that crude chlorophyll (i. e., all the pigments) increases in amount with the season, and from February to March far more than from March to May; from that time on it seems about constant. The chlorophyll proper increases likewise and decidedly more than the xanthophyll. This, she suggests, may be due to the conversion of the xanthophyll into chlorophyll; but KOHL's experiments strongly antagonize such an explanation.—C. R. B.

Stock and scion.—At a meeting of the Botanical Society of France last March GRIFFON discussed the results of his numerous experiments in grafting during 1908,[40] and declared that, whatever the plants employed (Solanaceae, Leguminosae, Compositae), and whether the graft was simple or mixed, there was no trace of asexual hybridization, but further confirmation of the specific independence of the stock and scion.—C. R. B.

An abnormal Funaria.—DIXON[41] describes a plant of *Funaria hygrometrica* from Tonduff having the perigonial leaves fringed by a double row of protuberant and more or less flask-shaped cells which are supposed to function as reservoirs of water supplementary to the paraphyses for keeping the antheridia well supplied. —C. R. B.

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[37] BOODLE, L. A., On the anatomy of the Gleicheniaceae. Annals of Botany **15**:703. 1901.

[38] KERSHAW, E. M., Note on the relationship of the Julianiaceae. Annals of Botany **23**:336, 337. 1909.

[39] STEIN, CÄCILIE, Beiträge zur Kenntnis der Enstehung des Chlorophyllpigmentes in den Blättern immergrüner Koniferen. Oesterr. Bot. Zeits. **59**:231–245, 262–269. 1909.

[40] GRIFFON, E., Troisième série de recherches sur la greffe des plantes herbacées. Bull. Soc. Bot. France **56**:203–210. *pls. 3, 4.* 1909.

[41] DIXON, H. N., A remarkable form of *Funaria hygrometrica*. Bryologist **12**:49–51. *pl. 5.* 1909.

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THE BOTANICAL GAZETTE

November 1909

Editors: JOHN M. COULTER and CHARLES R. BARNES

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VOLUME XLVIII NUMBER 5

BOTANICAL GAZETTE

NOVEMBER 1909

SOME FUNGUS PARASITES OF ALGAE[1]

GEORGE F. ATKINSON

(WITH EIGHT FIGURES)

About twelve years ago I was giving considerable attention to the study of the parasites of the algae in the vicinity of Ithaca, N. Y. At that time I hoped that the investigations might eventuate in a monograph of the Chytridiales of the Cayuga Lake basin. The pressure of other investigations has almost completely interrupted these studies. Because of our limited knowledge of the occurrence and habits of these interesting fungi in North America, it has seemed to me desirable that the observations already made should be recorded, in the hope that this may stimulate a greater interest in these plants.

As a result of the studies three papers have already been published. An extended paper on the genus Harpochytrium in the United States was published in 1903,[2] a summary of which later appeared in the *Journal of mycology* in 1904.[3] A short note on the interesting behavior of the zoospores of *Rhizophidium globosum* while escaping from the zoosporangium was published in 1894.[4] This behavior related to the habit of their sensing or feeling the exit opening in the sporangium, which they do by means of pseudopod-like extensions of the protoplasm in different directions, after having come to rest on the inside of the zoosporangial wall. In case they happen to come to rest close by the exit they "feel" it by one of the pseudopods, and slide out.

[1] Contribution from the Department of Botany, Cornell University, No. 133.

[2] ATKINSON, G. F., The genus Harpochytrium in the United States. Ann. Myc. 1:479–502. *pl. 10.* 1903.

[3] ———, Note on the genus Harpochytrium. Jour. Myc. 10:3–8. *pl. 72.* 1904.

[4] ———, Intelligence manifested by the swarm-spores of *Rhizophidium globosum*. BOT. GAZETTE 19:503, 504. 1894.

In case they are distant from the exit, not finding it they round up into the motile zoospore form again and swarm around in the zoosporangium for a time, and coming to rest make another trial. It is evident that when the zoosporangium is filled with the zoospores the latter will escape quite rapidly for a time because so many of them are crowded successively against the exit. As the numbers diminish there is greater freedom for swarming. The zoospores then swarm around and around in great circles inside the wall of the zoosporangium, now and then coming to rest and feeling around for the exit. This same behavior has been observed in the case of a number of other species. In addition to this a quite remarkable phenomenon was observed in the case of another species, *Rhizophidium brevipes*, which will be described below.

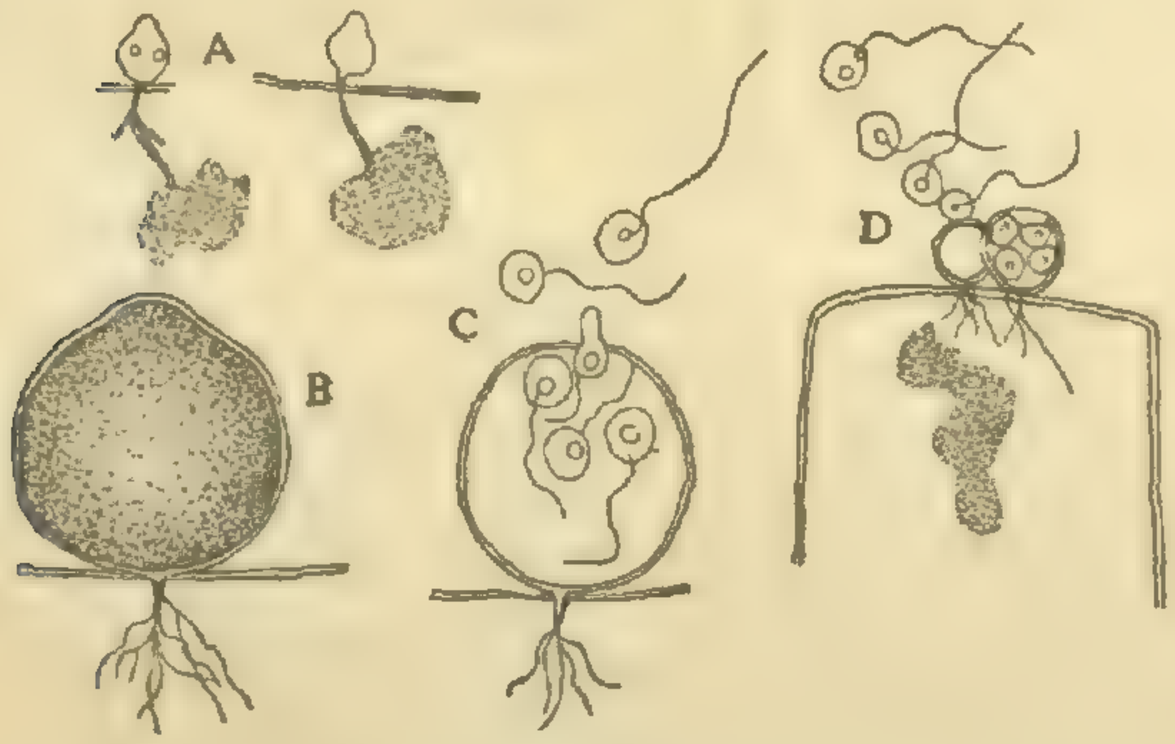

FIG. 1.—*Rhizophidium globosum* (A.Br.) Schröt. *A* young plant, shortly after germination of zoospores with germ tubes, penetration tube forming rhizoids; *B* mature plant ready to form zoospores; *C* zoospores escaping; *D* much smaller plants.

In the presentation of these observations I shall make no attempt to arrange the genera in any natural order of relationship, this matter being reserved for a future work. Since a number of species of Rhizophidium were studied I will begin with this genus.

RHIZOPHIDIUM BREVIPES

This was collected in a pool beyond Forest Home, N. Y., a little more than one mile from Ithaca. It was attached to the wall of a fruiting cell of *Spirogyra varians*. The zoosporangium is oval with a small apical papilla. The wall shows two distinct layers, an outer rather thick one and an inner thin one. At the time of the maturity of the zoospores the papilla of the outer layer becomes gelatinized at the apex, forming a minute opening about 4 μ in diameter.

One very characteristic feature of this species is the very rudimentary condition of the rhizoids. The very slender branched rhizoids so characteristic of *R. globosum* and other species appear to

be absent. The penetration tube of the zoospore forms a short stalk, which projects but a short distance within the cavity of the host cell. This rudimentary condition of the rhizoids recalls that of *Harpochytrium hedenii* Wille, though in the latter it penetrates only the outer lamella of the wall and flattens out in the form of a disk in the middle lamella, or merely penetrates a thin layer of slime on the host and flattens out on the outer wall (ATKINSON 1903). This penetration tube serves also as the absorbent organ for such food as the plant obtains from the fluids within the gametangium of the host surrounding its zygospore. The zoosporangium measures 21–24 μ, while the zoospores are about 3 μ in diameter, with a single cilium and a prominent oil drop.

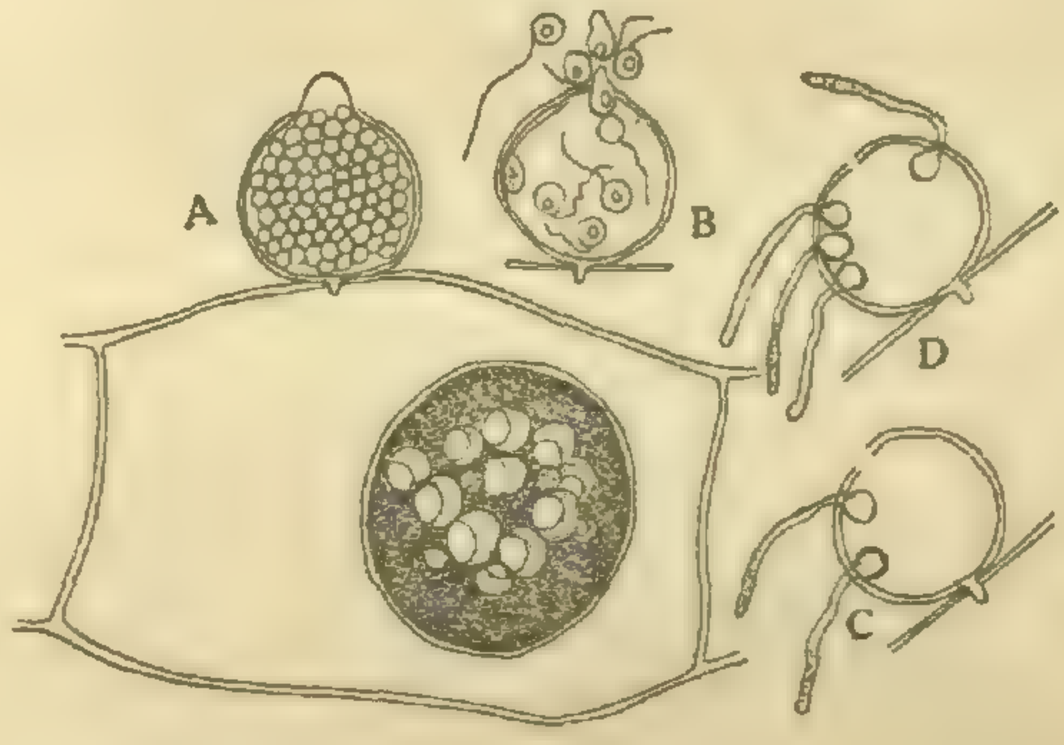

FIG. 2.—*Rhizophidium brevipes* Atk. *A* mature zoosporangium crowded with zoospores, outer layer of wall at exit pore dissolving, inner layer projecting as a papilla before the rupture, plants attached to a gametangium of Spirogyra containing zygospores; *B* zoospore escaping; *C* two zoospores, unable to escape by exit pore, have germinated and are attempting to escape through germ tube, protoplasm in the apex of the tube; *D* same later, showing how the zoospores retreated from the first tubes and after swarming around for the second time attempted to escape again by the germ tubes.

The species was first observed in a cell preparation which was made on April 24, 1895, at about 4 P. M. The zoosporangium was mature and the protoplasm finely granular. In the course of half an hour from the time of the first observation, the granules began to arrange themselves in numerous small groups, the beginning of the formation of the zoospores. Very soon these began to disappear and were gradually replaced by a prominent oil drop for each group of granules. Each oil drop was surrounded by a hyaline mass of homogeneous protoplasm. The preparation was watched for the greater part of the time from 4 P. M. until 6 P. M., and fresh water was frequently drawn in, in order to hasten the development and maturity of the zoospores. At this time it appeared that the zoospores would not become mature under an hour, and the preparation was placed in a moist chamber until 7 P. M. It was then examined and fresh water added. The apical portion of the outer wall was dissolved;

soon the inner wall at this point became ruptured, and precisely at 7 P. M. the zoospores began their escape. The number of zoospores was very large and they were packed so tightly in the zoosporangium that it was impossible for them to make other than slight amoeboid movements. Those at the apex slowly moved through the opening one or two at a time, so that the number could be readily counted. In passing through the opening the zoospore puts forth a stout hyaline projection which feels and leads the way, and then the body slowly moves through. On the outside, at the mouth of the zoosporangium, each zoospore rests for a short time, during which a few plastic movements are exhibited; then it rounds off and darts away.

When 50 or 60 zoospores had made their escape in this way the space in the zoosporangium was not so crowded, and a few of those at the center began active swarming movements, while those at the periphery of the sporangium were quiet or only exhibited plastic movements, and those nearest the opening continued to escape in the manner described. This continued until there were very few still within the zoosporangium. The remainder of the zoospores were all swarming, and at times some of them would come to rest on the side of the wall, put out by amoeboid movement a short pseudopodium, and feel evidently for the opening. Not finding it they would swarm around within the zoosporangium again, and again come to rest, maneuver for the opening, and on finding it escape. This manner of finding the opening is the same as that described for *R. globosum* (ATKINSON 1894), but as the opening is larger than in that species, the body of the zoospore does not become constricted at the passage. At 7:18 P. M. all but four of the zoospores had made their escape, and in four minutes more two of these had escaped, leaving two still within the zoosporangium. These were watched for an hour longer, and they divided the time in swarming and feeling for the exit, but were unable to escape; though several times they located themselves directly at the opening, they seemed to be insensible of it.

The preparation was placed in the moist chamber and left for the night. On the following morning both of the zoospores were found located on one side of the zoosporangium and each had germinated.

The slender germ tube, having penetrated the wall of the zoosporangium, extended in a tortuous course for a distance of 15–20 μ. The preparation was examined several times during the day. Since one of the zoospores was located not far from the wall of the spirogyra, it was hoped that the thread would find the cell and enter it, inasmuch as it was pointed in that direction. The preparation was left in this condition during another night, the slender tube of the zoospore nearest the spirogyra cell not yet having reached it. On the following morning the preparation was examined again, and I was surprised to find that one of the zoospores was on the other side of the zoosporangium. Close examination showed that both had moved during the night. Not being able to find any suitable nourishment, the protoplasm in each germinating cell had migrated back from the inclosing membrane into the zoosporangium, formed a zoospore, and had passed through a second swarming period. Then coming to rest again they had germinated, the germ tube of each having again penetrated the wall of the sporangium and formed a slender tube 15–20 μ long. The preparation was kept for a day longer, but the zoospores did not form again.

This behavior of the zoospores is quite interesting, since it manifests not only a sort of "ingenuity" in the attempt to escape from the zoosporangium, but, what is more important, the tendency, under certain conditions of existence which prevent the zoospore from seeking a host cell by the normal planetic method, to migrate in the form of a true mycelial tube, which is quite different from the very delicate slender absorbent rhizoids normally developed from the germ tube in other species of the genus.

Rhizophidium brevipes, n. sp.—Zoosporangia oval, 21–24 μ in diameter, with a papilla at the apex, in which the exit pore is formed. Rhizoidal apparatus very rudimentary, consisting of the short, blunt, unbranched entrance tube of the zoospore, which projects but a very short distance beyond the inner lamella of the wall of the host. Zoospores oval with an oil drop, uniciliate, 3 μ in diameter.

On walls of gametangia of *Spirogyra varians* in which zygospores are formed. Vicinity of Ithaca, N. Y.

Zoosporangiis ovatis, apici uno, brevi, papilliformi ostiolo, basi, uno brevi, non ramoso tubulo. Zoosporis ovoidiis, 3 μ latis, una guttula hyalina et uno cilio praeditis.

RHIZOPHIDIUM SPHAEROCARPUM

Rhizidium sphaerocarpum Zopf, Nova Acta Leop.-Carol. Deutsch. Akad. **47**:202. *pl. 19. figs. 16–27.* 1884.

Rhizophidium sphaerocarpum A. Fischer, Rabenh. Krypt. Flora Deutschl. Oesterr. u. Schweiz 2 Aufl. **4**:95. 1892.

What I have taken to be this species occurred in abundance on threads of *Mougeotia parvula* collected in a gutter on Thompson St., Ithaca, N. Y., April 7, 1895. The zoosporangia are oval in form and vary considerably in size, the larger ones measuring 16–18×18–20 μ, while the smaller ones are about 10×11 μ. They occur singly or in groups of two to four, the smaller ones more commonly in groups and the larger ones rarely so. The rhizoids are very much reduced, consisting of a few very short branches from the short entrance tube.

The wall of the zoosporangium consists of two lamellae, an outer stout lamella and a thin inner membrane. This is very well seen in the dehiscence of the zoosporangia. At maturity the apex of the outer wall of the zoosporangium dissolves, forming a large circular opening for the exit of the zoospores. Through this the inner membrane projects in the form of a short broad papilla by the swelling of the epiplasm. The final rupture of this membrane sets the zoospores free, and the large opening permits their rapid escape. The exit pore measures 5–6 μ in diameter in the larger forms, and in the smaller ones is proportionately larger, being 4–6 μ in diameter, some of the smaller zoosporangia appearing like cups when open. The zoospores are oval, possess a long cilium and a prominent oil globule, and measure 1.5–2 μ in diameter. In germination, if the germ tube is directed toward the wall of the host cell, it penetrates the wall, and then the zoospore enlarges to the size of the mature plant, becoming the zoosporangium. If, however, as sometimes happens, the germ tube is directed away from the wall, or along its surface, it may grow to a considerable distance, sometimes reaching a length of 30 μ. The zoosporangia sometimes grow so as to appear attached by their side instead of by their base, and the opening is then at the side (*fig. 3, C*).

In nearly all of the larger specimens (which may prove to be a different species) the effect on the host cell was quite remarkable. The host cell not only becomes considerably larger near the middle,

but is very much elongated. Frequently at the middle it is not much larger, while on each side an enlargement occurs (*fig. 3, D, G*). The stimulus which causes the increase in the length and size of the cell also increases the development of the chromatophore. When this influence ceases the chromatophore begins to degenerate, the color

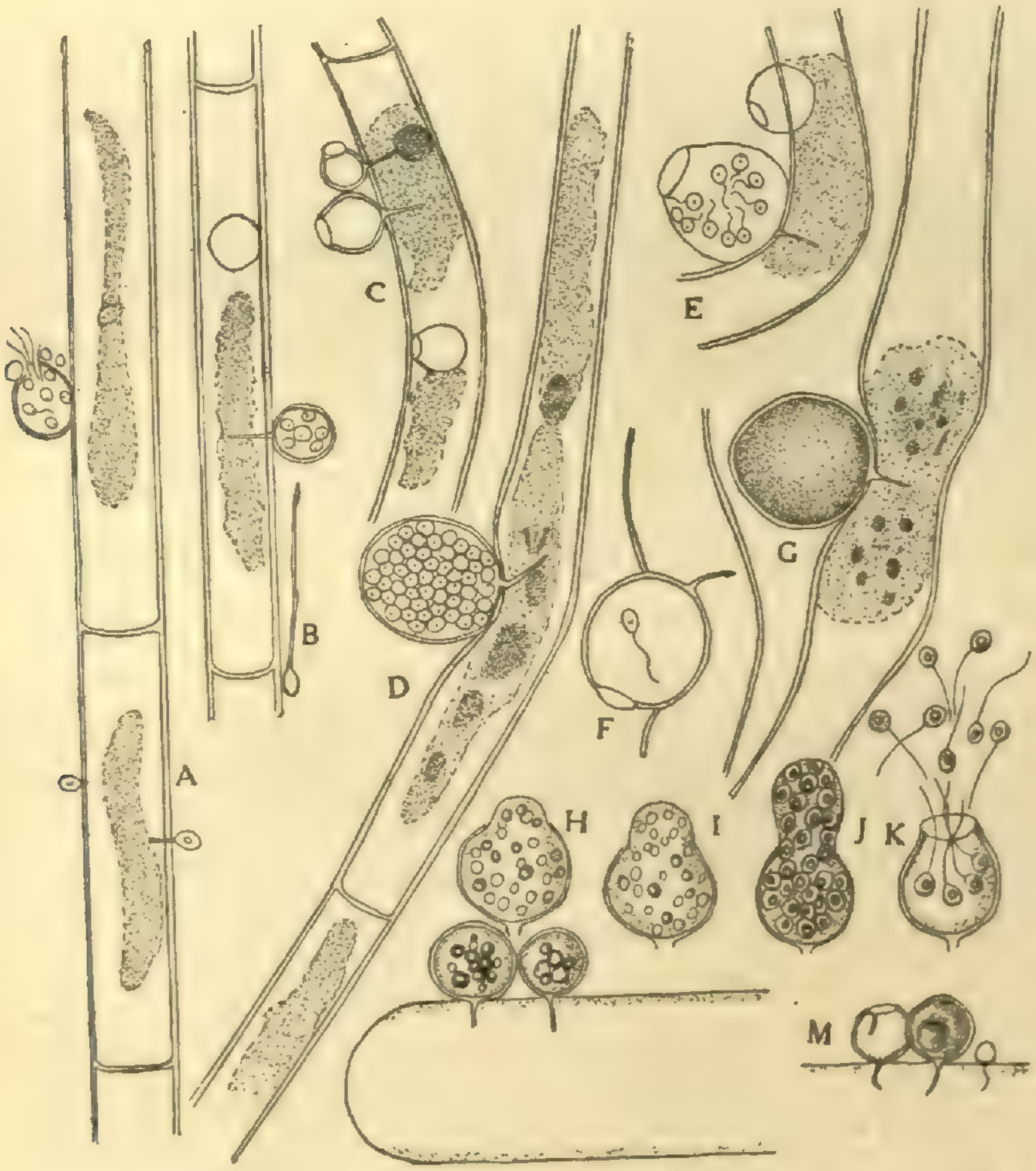

FIG. 3.—*Rhizophidium sphaerocarpum* (Zopf) Fisch. *A* filaments of Mougeotia showing entrance of germinating zoospores, and mature plant with zoopores escaping; *B* zoospore germinating, with germ tube growing parallel with the host thread, mature plants also attached to the host; *C* mature empty zoosporangia, showing one with the penetration tube on the side; *D* mature plant, large form, showing hypertrophy and curving of host cell; *E* mature plant with escaping zoospores, showing strongly curved host cell; *F, G* showing hypertrophy of host cell; *H–M* different stages in the opening of the zoosporangium and escape of the zoospores; *A–G* original; *H–M* after ZOPF.

fades, the structure breaks down, and the processes of dissolution separate it into small particles with the pyrenoids more distinct, all coalescing into an amorphous mass, which gradually flows toward the middle of the cell where the sporangium is located. This may be due partly to the fact that the cell of the host is larger at that point, as well

as to an influence which the parasite exerts upon it, for it is possible that the crowding of the chromatophores from each side toward the middle may cause the two enlargements near the middle with the constriction between them (*fig. 3, G*). Not only does the influence of the parasite excite these changes in the host cell, but it also causes the cell to become more or less strongly arched at the point of insertion of the sporangium, the sporangium being in the concavity of the arched thread. This effect has only been noted in connection with the larger forms, except where the smaller ones are several in a group, and here the curving of the thread is slight in comparison with that which exists in the case of the larger sporangia. ZOPF (*l. c.*) does not mention any similar hypertrophy of the host cell caused by this species, nor is it shown in his illustrations. He figures the plant on Spirogyra. A. FISCHER (*l. c.*) reports it on various filamentous Conjugatae and Oedogoniaceae, but does not mention any hypertrophy of the host.

Germinating zoospores have been observed in this species in which the germ tube may become directed away from the host and develop a mycelial tube 15–20 μ in length.

RHIZOPHIDIUM MINUTUM

This species was collected on *Spirogyra varians* in a pool beyond Forest Home, N. Y. (about one mile from Ithaca), April 23, 1895. It frequently accompanies Lagenidium, but also occurs independently of it. The zoosporangia are sessile, very small, obpyriform or flask-shaped. Thus the species belongs to the *longata* section of the genus. The zoosporangia are 5–6 μ in diameter. At the base are a few slender rhizoidal threads which extend a short distance in the host cell content. The form of the zoosporangium thus presents a broad and prominent apical papilla, the end of which becomes gelatinized at maturity of the zoospores, forming a circular exit pore. The zoospores are few in number, two to four in the cases observed. They measure 2.5 μ in diameter, and are provided with one cilium and a prominent oil drop.

FIG. 4.—*Rhizophidium minutum* Atk. Elongated zoosporangium, rhizoids, and zoospores.

Rhizophidium minutum, n. sp.—Zoosporangia obpyriform or flask-shaped, broadly papillate, 5–6 μ in diameter, sessile with a few slender rhizoidal filaments

at the base in the host cell. Apex opening by a single pore. Zoospores two to four in a zoosporangium, oval, uniciliate, with a single oil drop, 2.5 μ in diameter.

On *Spirogyra varians* in the vicinity of Ithaca, N. Y.

Zoosporangiis obpyriformibus, 5–6 μ latis, apice una lata papilla, basi filamentis brevibus radiciformibus. Zoosporis ovoidis, 2.5 μ, una guttula hyalina et cilio simplici attenuato praeditis.

LAGENIDIUM RABENHORSTII[5]

This species was first found in a species of Spirogyra in a stream of slow-running water in the valley south of the city of Ithaca. It has since been found in various species and appears to be quite common. The fungus attacks the vegetative cells and those just about to conjugate. The zoospore enters the cell wall by a small perforation, the slender entrance tube growing usually nearly to the center of the host cell, where it enlarges to the size of the vegetative thread of the parasite. The general course of the threads is parallel with the axis of the spirogyra cell, though frequently tortuous and curved, with here and there short branches. The threads are usually stout, varying in diameter from 3 to 8 μ. At the ends of the cell of the host they curve around and frequently extend back to the other end, one to three and four threads thus lying in a single cell. The protoplasm possesses numerous highly refringent granules and there are also vacuoles at short distances. In other cases the thread may be strongly curved, or even coiled at various points within the end of the cell.

The chromatophores of the spirogyra are broken down and usually adhere to the threads of the fungus here and there, giving portions of them a green appearance in the early stages, and later a greenish brown as the chlorophyll becomes more and more disorganized. All traces of the chlorophyll at length disappear, and the fungus, lying in a disorganized mass of transparent protoplasm, is then seen distinctly throughout its entire length. In other cases the fungus thread may be permanently soiled by the brownish matter of the disorganized chlorophyll.

The exit tubes are developed from the ends of the threads, or from the ends of the short lateral branches, or from the side of the main

[5] ZOPF, W., Ueber einen neuen parasitischen Phycomyceten aus der Abtheilung der Oosporeen. Bot. Verein Prov. Brandenburg **20**:77–80. 1878. See also, Zur Kenntniss der Phycomyceten (Zur Morphologie und Biologie der Ancylisteen und Chytridiaceen). Nova Acta Kais. Leop.-Carol. Deutsch. Akad. Naturf. **47**:143–236. *pls. 12–21.* 1884.

thread itself, in the latter cases usually arising from the outer convexity of a curved portion of the thread. The exit tube is about 2 μ in diameter and projects but a little way outside of the wall of the host. The tube develops quite rapidly. In observations on cell cultures where I have been watching for the migration of the protoplasm through the exit tube, forming ones have been observed to develop in two to four hours time. The end of the tube becomes gelatinized and the protoplasm moves in a quite rapid stream through the tube, the small spherical mass of protoplasm at the mouth of the tube growing rapidly in size until all the protoplasm has passed through, which takes only a few seconds. In a few moments rotary motion begins, slow at first. Soon the constriction of the mass occurs in lines over the surface, in such a way as to divide the surface into 2–8 portions according to the size of the mass and the number of the zoospores to be formed. In this species I have not observed any inclosing membrane, nor any evidence that such a membrane exists, though ZOPF (*l. c.*, p. 149, 1884) describes and figures one. The constriction goes on from the surface toward the center, and soon the cilia are developed, their slow waving aiding in the slow but perceptible oscillatory motion of the mass. Plastic movements of the mass and the developing individuals also accompany the other movements. When division has become nearly complete, small portions frequently become constricted from the ends of the ovate to reniform individuals, the constriction becoming deeper and deeper, until in many cases the small portion becomes separate from the larger mass (*fig.* 5, *C*). These can be seen to be separate from the larger ones by the gliding motion of the zoospores over each other, and over the smaller bodies. The movements become more and more active, occasionally one zoospore pulling strongly in

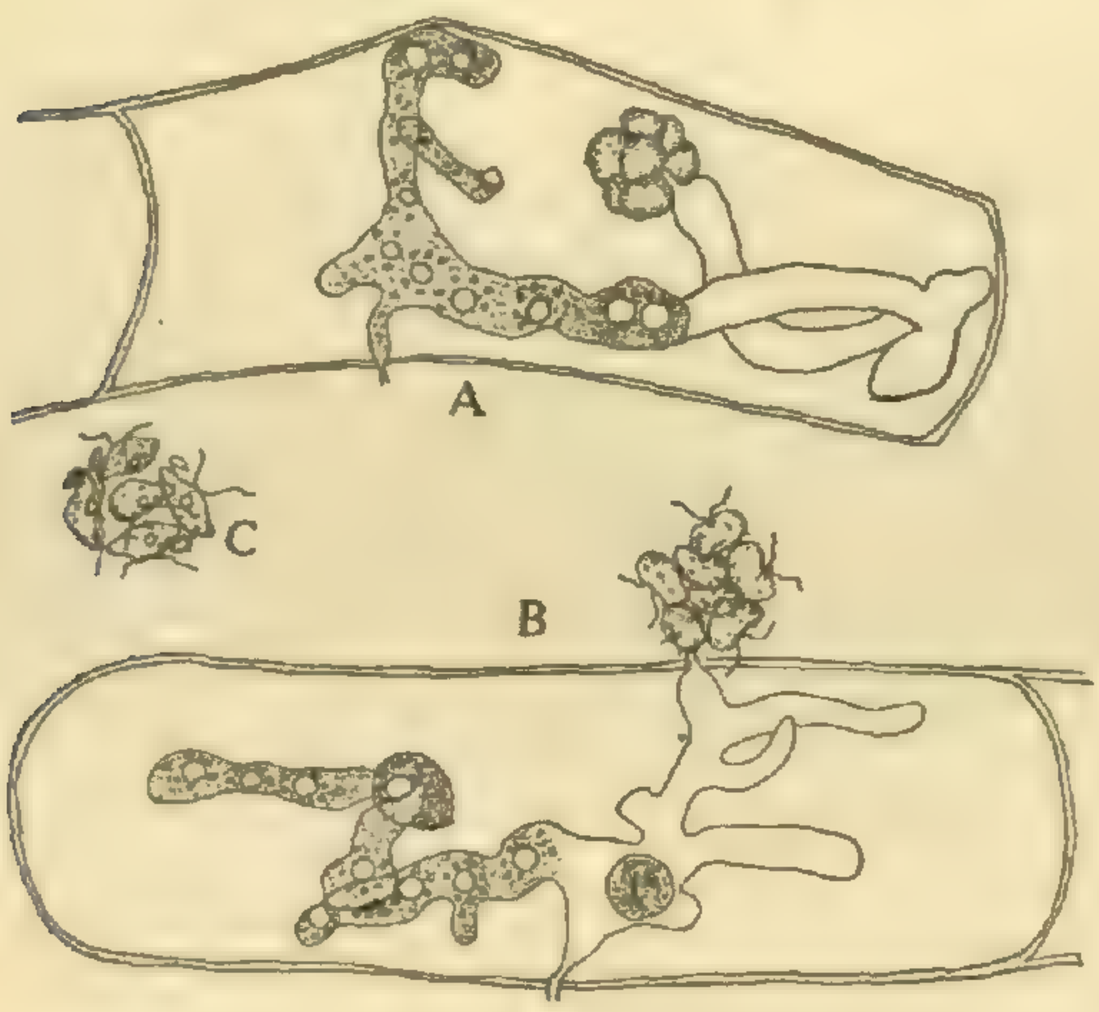

FIG. 5.—*Lagenidium rabenhorstii* Zopf. *A* thallus and prosporangia, which are sections of the thallus; *B* zoospores forming at the apex of the exit tube; *C* zoospores and small particles separated from them.

one direction and separating itself from the group by several millimeters; then it is drawn back again to its fellows as if by a retractile cord; then another will separate in the same manner, only soon to be drawn back again. This pulling away and returning becomes more and more accentuated each time, until finally one of the zoospores makes its permanent escape, leaving the others to struggle still for freedom. One by one, or two or more at a time, they escape in this manner and whirl away. This mode of escape would indicate that there is no inclosing membrane.

Before the escape of the zoospores, in cases where small portions of the zoospores have become separated, they may fuse or conjugate with the large ones again, but whether with the same one from which they were separated or with a different one could not be determined, since the gliding and rotary movement of the individuals and the mass would prevent absolutely the following of the separate ones through their various evolutions. DeBary[6] (p. 37, 1881) first observed a separation of portions of the protoplasm and their fusion again with the parent masses in the formation of the oospores of the Saprolegniaceae, and it has since been observed in the oospores by Hartog[7] (p. 24) and Humphrey[8] (p. 90). It was first observed in the zoospores of the Saprolegniaceae by Rothert[9] (1887, also p. 322, 1890; see also Hartog, *l. c.*). When fusion did not take place before the zoospores made their escape from the group, the smaller portions would not then fuse with the larger ones, even if they came by accident in contact with them, so far as I was able to observe. In other cases the small portions of protoplasm might become partially separated from the larger ones, and the zoospores escape with a minute appendage attached either at one of the extremities or upon

[6] DeBary, A., Untersuchungen über die Peronosporeen und Saprolegnieen und die Grundlagen eines natürlichen Systems der Pilze. Beitr. Morph. u. Phys. der Pilze **4**:1–145. *pls. 1–6.* 1881.

[7] Hartog, M. M., Some problems of reproduction. Quart. Jour. Micr. Sci. **33**:1–79. 1892.

[8] Humphrey, J. E., The Saprolegniaceae of the United States, with notes on other species. Trans. Am. Phil. Soc. **17**:63–148. *pls. 14–20.* 1893.

[9] Rothert, W., Die Entwickelung der Sporangien bei den Saprolognieen. Beitr. Biol. Pfl. **5**:291–349. *pl. 10.* 1890. This paper first appeared in Polish in the Proceedings of the Cracow Academy **17**:———. 1887.

the more convex side, where it gave the zoospore the appearance of being a "hunch back." Such zoospores would perform very curious evolutions in their vain efforts to throw off the offending appendix, but in no case observed did this separation take place after the zoospores had separated from the group at the mouth of the exit tube. The number of zoospores from a single zoosporangium is 2–8, as stated above, or possibly a few more in some cases, the number depending upon the size of the sporangium.

In one case observed, where the zoospores seemed to be rather sluggish in their development and the movements not so active, the form was not so pronouncedly reniform, but more nearly oval, and after separating very little active movement took place, the individuals soon rounding off as they do when ready to germinate. Two such zoospores soon came in contact and immediately fused into a larger one, the fusion taking place in about ten seconds. The further fate of these zoospores was not followed. This fusion may be due to the peculiar conditions of environment, perhaps to the want of fresh water in the limits of the cell culture. From some observations made on the developing zoospores under similar conditions, it appears possible that it may be due to the variations of tension existing between the individuals and the original mass of protoplasm, the tension of the entire mass being directed toward keeping the mass intact and the tension of the individuals tending to separate the masses into smaller individuals. Two cases were observed which had progressed to some extent toward the formation of the zoospores. In one case there were two zoospores forming and in the other case four zoospores were forming from the spherical protoplasmic mass, which had collected at the end of the exit tube after passing from the sporangium. In each case the zoospores were about one-third formed. The preparation had been in the cell culture for two days and the water had been replenished a few times, as it had partially evaporated, by running fresh water under the cover from the edge, the cell cultures being made simply between the cover glass and the glass slip and not in a ring-cell, or VAN TIEGHEM cell. The oxygen thus accessible to the organism was very small, though this did not seem to hinder the development during study, if water were added every few moments, as would be necessary when the preparation was not protected in a

moist chamber. While these two developing groups of zoospores were under observation, the water, which had not been changed for some time, slowly evaporated, so that a portion of it was removed from under the cover. At this time it was noted that the dividing protoplasmic mass, where there were two forming zoospores, was fusing again, and soon the mass was spherical, with no sign of the division which up to this time was quite marked, and showed all the phenomena of movement and formation of cilia which accompany the normal development of the zoospores, except that the movements were not so active. Very soon also the four forming zoospores in the other group were fusing and the movements had likewise ceased. The fusion in this case also continued until there was no trace of the forming zoospores, the mass was again spherical, and movement had ceased.

Thinking this might be due to the want of fresh water, some was quickly run under the cover glass, and the observation was renewed. The smaller protoplasmic mass burst on the absorption of the water, so great was the tension, but the larger one soon began slow rotary movement again and the constrictions appeared a second time, indicating the formation of four zoospores. This time the division proceeded, accompanied by all the phenomena of the formation of the zoospores noted in the normal cases, until the zoospores were complete and whirled away.

This would suggest that there were two opposing tensions in the formation of the zoospores, one individual and under normal conditions the stronger, and the other belonging to the mass and the weaker. When the conditions favorable for the formation of the zoospores ceased, the individual tension lessened to such a degree that it was lower than that of the mass, and the separating smaller portions were drawn together again in the common larger mass. These two opposing tensions might possibly explain the peculiar behavior of the zoospores when they still remain closely associated in the group, now partially separating and again coming close together, continuing this process of coquetting until the individual tension is strong enough to free them, in the case of those where there does not seem to be any inclosing membrane. The tension of the entire mass may possibly be somewhat similar to the force called adelphotaxy

by HARTOG[10] (p. 216) in the case of the escaping zoospores of Achlya and Aphanomyces.

In some of the cell cultures in which numerous zoospores of Lagenidium were developing, there were several sun animalcules (*Actinophrys sol*). Some of these were so full of zoospores which they had caught, that they appeared to be a rounded collection of the zoospores, the convex outer portion of the zoospore only showing, and the entire surface of the Actinophrys appearing like a piece of mosaic made up of these unfortunate creatures. The delicate arms or rays of the Actinophrys radiated for a distance of 20–30 μ, and in a large number of cases which were observed, whenever a zoospore passed within reach of these rays, no matter how swiftly it was moving, it was suddenly paralyzed and halted; then the reniform shape gradually became spherical and it was slowly drawn by "invisible threads" to the body of the Actinophrys and slowly wedged its way between those which had preceded it.

LAGENIDIUM AMERICANUM

Another species of Lagenidium, which seems to differ from any described, was found in various species of Spirogyra (*S. varians*, *S. calospora*, and *S. insignis*) collected in a pool beyond Forest Home, April 11, 1895. It is parasitic in the zygospores and conjugating cells of the spirogyras, but has not been observed to develop in the vegetative cells. The vegetative phase of the fungus consists of strongly curved and coiled tubes, 4–8 μ in diameter, the size varying so that the contour and diameter of the threads is very irregular. It is profusely branched, and has also numerous short branches. It frequently fills the zygospores so completely that the individual course of the thread can be followed for only a short distance. At maturity the tube, as in the other species, is separated into segments of various lengths by the development of cross-walls, forming the zoosporangia. Frequently there is a constriction at the septum. Numerous zoosporangia are developed in a single zygospore. The exit tubes are slender, measuring about 2 μ in diameter, and of variable length according to the direction in which they emerge from the zygospore and the wall

[10] HARTOG, M. M., Recent researches on the Saprolegnieae. Annals of Botany 2:201–216. 1888.

of its parent cell. If the zoosporangium is located at the periphery of the zygospore near the middle, the tube is quite short. In the case of the zoosporangia which are located at the ends of the zygospores, the length of the exit tube is considerably longer, even when it is directed from the start perpendicularly to the wall of the parent spirogyra cell. In many cases, however, the exit tube from the zoosporangia which lie at the ends of the zygospore starts out nearly parallel with axis of the spirogyra cell, or at some angle between this and the perpendicular. In such cases the exit tube may be very long and tortuous, frequently passing into the adjacent spirogyra cell and finally emerging through the wall of the latter.

The protoplasm, with numerous small highly refringent granules and a number of vacuoles, presents practically the same appearance as in the case of the other species. It passes in a rapid stream through the exit tube and collects in an irregularly spherical mass at the extremity, from which in some cases it soon becomes free and floats to a short distance. In ten minutes from the passage slight rotary movements begin, the mass turning a short distance in one direction, and then in a moment in the other direction, the movement becoming faster and repeated at shorter intervals.

In some cases the cilia can be seen as delicate rays, even before the simultaneous constriction of the mass begins and they are slowly lashing. In other cases they are observed very soon after the constriction of the mass begins which outlines the surface of the individual zoospores. No inclosing membrane was observed, and it seems impossible that it should exist if the cilia can extend for such a distance from the mass. As the division proceeds the reniform shape of the individuals becomes more and more pronounced, and the movements become more and more violent. The individuals divide the time in a quivering motion followed by the oscillatory movements and the gliding over one another until they are apparently separate, now one pulling off a short distance, then returning, and so on, until they make their escape one after another or several at a time. After separating from the group, movement in space is rather slow, as if they were uncertain how to use the freedom gained. The individual oscillates and glides around in various curves, then becomes nearly stationary and quivers for a moment; then, one end remaining

stationary, the other executes in succession several jerks or nods as if striking at something; then it enters upon another series of evolutions. After a short time thus engaged, the activity of the zoospore increases, the field covered by its evolutions becomes larger, and it is lost from the field of the microscope. In some cases which were timed the zoospores were separate in 25–30 minutes from the time the

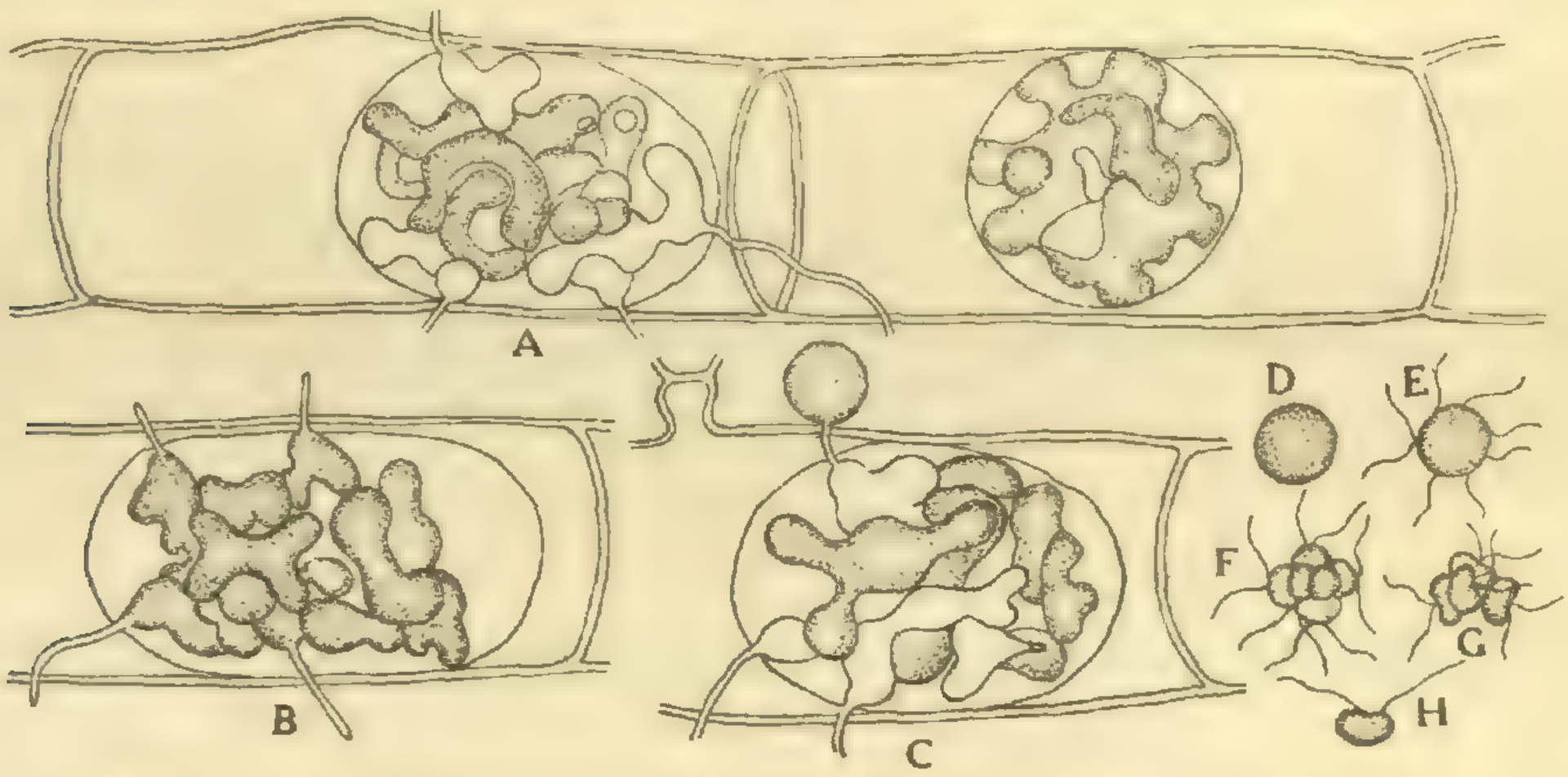

FIG. 6.—*Lagenidium americanum* Atk. in zygospores of Spirogyra. *A*, *B* some empty and some mature prosporangia; *C* from one prosporangium the protoplasm has just issued from the exit tube; *D* plasma sphere or zoosporangium proper free from the exit tube and floating in water; *E* formation of cilia where a zoosporangium is swimming around as a multiciliate spore; *F*, *G* different stages in formation and separation of zoospores, the groups swimming around like a pandorina colony; *H* mature and separate zoospore.

protoplasm passed from the exit tube. In those cases where the plasma vesicle at the end of the tube becomes free and floats away, the cilia develop on its surface, and it at first presents the appearance of a multiciliate spore. Later the divisions occur in such a way as to leave two cilia with each forming zoospore, and the colony then resembles a pandorina colony.

Lagenidium americanum, n. sp.—Plant body in the form of irregular tubes, much branched, curved, and of unequal diameter, confined within the host zygote, 3–8 μ in diameter. Exit tubes slender, short, 2 μ in diameter, varying in length but extending a short distance outside of the gametangium wall, of equal diameter from the point where they pierce the wall of the zygote to the outer free end, thus differing from the exit tubes of *L. entophytum* (Pringsh.) Zopf. Exit tubes arising from the end of hyphae or branches, or from the convex side of curved or enlarged portions. Zoosporangia of varying size, irregular and often branched, formed from segments of the thallus. Plasma sphere at the end of the exit tube often becoming free and floating away (the cilia then forming on the outside of the

sphere), and finally dividing into the individual zoospores, the whole resembling a pandorina colony, but at last the zoospores separating. Zoospores reniform, laterally biciliate, 4–6×5–7 μ.

In zygospores of *Spirogyra varians*, *S. insignis*, and *S. calospora*, Ithaca, N. Y.

Mycelio inequali, curvato, ramulo irregulare instructo, 3–8 μ lato. Zoosporangiis irregularibus, saepe ramulosis. Ostiolo tubiformi tenuissimi aequali, 2 μ lato. Prosporangiis ramulosis sectionibus mycelii formatis. Zoosporangiis vesiculosis, saepe deciduis, ciliis ornatis, ut coenobio Pandorinae natantibus, postremo divisis, zoospores formantibus. Zoosporis fabaeformibus, laterale biciliatis, 4–6×5–7 μ. Hab. in zygosporis Spirogyrae, Ithaca, N. Y.

PHLYCTOCHYTRIUM PLANICORNE

This genus, *Phlyctochytrium* Schroeter,[11] differs from Rhizophidium in the presence of a swelling on the penetration tube, just inside the host cell wall, from which the delicate nutritive rhizoids grow. *Phlyctochytrium planicorne* has been found quite frequently in company with *Lagenidium americanum*, in the cells of *Spirogyra varians*, from the pool beyond Forest Home (near Ithaca). The zoosporangium is a little broader than long, measuring 6×8 μ, and is broadest in the middle. At the apex it is provided with four plain dentate processes around the exit pore. These dentate processes are characteristic of one section of the genus (*Dentigera* ROSEN,[12] SCHROETER, *op. c.*, p. 79). The endophytic bladder-like base of the plant is separated from the epiphytic zoosporangium by a constricted portion at the point where the penetration tube passes through the cell wall of the host. This base is about 3 μ in diameter, and from it radiate several slender branching threadlike rhizoids. No zoospores have as yet been observed. While it very frequent'y accompanies Lagenidium, it is by no means confined to the cells affected by the fungus, being found on other cells also.

FIG. 7.—*Phlyctochytrium planicorne* Atk.

Phlyctochytrium planicorne, n. sp.—Zoosporangium broadly elliptical, 6×8 μ, armed at the apex with four plain teeth. Endophytic vesicle globose, 3 μ in diameter, with several radiating branched slender rhizoids.

On cells of *Spirogyra varians*, often accompanying *Lagenidium americanum*, near Ithaca, N. Y.

[11] SCHROETER, J., in ENGLER & PRANTL, Pflanzenfam. 1[1]:78. 1892.

[12] ROSEN, F., Ein Beitrag zur Kenntniss der Chytridiaceen. Beitr. Biol. Pflanzen 4:253–266. *pls. 13, 14.* 1887.

Zoosporangio ellipsoideo, 6×8 μ, apice 4 cornuis ornato. Cellula inferiori 3 μ lata. Hab. in *Spirogyra varianti*.

PHLYCTOCHYTRIUM EQUALE

This species inhabits the cells of Spirogyra, having been found in the cells of *S. insignis*, collected in the pool beyond Forest Home, April 17, 1895. The epiphytic sporangium and the subsporangial endophytic base are about equal in size, being spherical in form and about 6 μ in diameter. The mouth of the sporangium appears to have two small teeth, which may be the walls of a short canal. From the base of the subsporangial part of the plant are several branching rhizoid-like threads. Zoospores have not been observed.

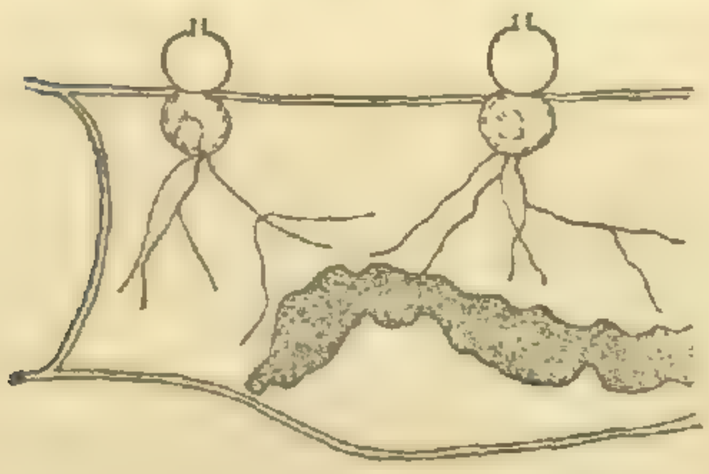

FIG. 8. — *Phlyctochytrium equale* Atk.

Phlyctochytrium equale, n. sp.—Zoosporangium globose, sessile, about 6 μ in diameter. Subsporangial base equal in size, with several long branched rhizoid filaments from its base.

Zoosporangio globoso, sessili, 6 μ lato, basi subsporangiali aequali, rhizoideis filamentibus ramulosis praedito. Hab. in *Spirogyra insigne*.

A few other species have been observed but not studied further than for their identification. They are as follows:

Lagenidium enecans Zopf (p. 154, 1884), in *Stauroneis phoenicentron* and *Cymbella lanceolatum*, in swamps near Freeville, N. Y., May 5, 1895.

Entophlyctis bulligera (Zopf) Fischer (*Rhizidium bulligerum* Zopf, p. 195, 1884), in *Spirogyra insignis* in pool at Forest Home, N. Y., April 17, 1895.

Rhizophidium ampullaceum (A. Br.) Schroeter, on sterile threads of Oedogonium, Freeville, N. Y., May 4, 1895.

Ectrogella bacillariacearum Zopf (p. 175, 1884), in diatoms, south end of Cayuga Lake, Ithaca, N. Y.

CORNELL UNIVERSITY

MITOSIS IN SYNCHYTRIUM WITH SOME OBSERVATIONS ON THE INDIVIDUALITY OF THE CHROMOSOMES[1]

ROBERT F. GRIGGS

(WITH PLATES XVI–XVIII)

The nuclei of *Synchytrium decipiens*, a leaf parasite of the hog peanut, were shown in previous papers to be derived very largely by amitosis. Several sorts of amitosis were observed, two of which, nuclear gemmation and heteroschizis, differ considerably from the ordinary process of amitosis. The nuclei derived by these direct divisions were shown to be persistent, and some of them were observed to divide by mitosis. This with other facts led me to the conclusion "that the nuclei derived by these processes of amitosis are normal, and that they with their descendants become the functional nuclei of later generations, capable of perpetuating the species" (**5,** p. 134). The purpose of the present paper is to describe the mitoses which follow, to correlate them with the amitoses, and to discuss the theoretical bearing of the facts thus presented.

I would here repeat my acknowledgments to my friend, Professor F. L. STEVENS, of the North Carolina College of Agriculture and Mechanic Arts, who furnished the material used in the investigation and gave the suggestions that originally aroused my interest in the problem. The sections were cut 2–10 μ thick and stained with Haidenhain's iron alum hematoxylin and with anilin safranin and gentian violet.

As in coenocytes generally, all the nuclei in a cyst pass into mitosis simultaneously and divide with equal rapidity, so that all of them are very nearly in the same stage throughout. This fact may be utilized for overcoming one of the most serious obstacles encountered in investigating the cytology of this plant—the difficulty of estimating the relative ages of the different structures in the absence of any

[1] Contribution from the Botanical Laboratory of the Ohio State University, No. XLIX.

indications external to the nuclei themselves—for those cysts which are of critical age usually contain short series of closely connected stages which frequently enable one to assure himself concerning difficult points, such as the formation of the spindle or the origin of the asters. Nuclei of widely different phase in the same cyst are very rare: *fig. 5* is a case where the small nuclei in the cyst were still in the vegetative condition while the large nuclei had passed into spirem; *fig. 35* shows a case where the small nuclei had reached late anaphase while the large nuclei were still in metaphase; *fig. 36* of the previous paper (**5**) is a case where the reverse was true, the small nuclei being in metaphase while the large ones were in anaphase. It would be a matter of great interest to ascertain the mechanism by which the nuclei are kept in phase. While not throwing much light on the nature of the stimulus, the behavior of newly segmented cysts is interesting in this connection. In such a cyst, long before the walls of the zoosporangia appear, while the segments are still separated from each other only by an exceedingly delicate plasmatic membrane, each nucleus has become entirely independent of the others; and one finds vegetative nuclei, metaphases, and telophases in adjacent segments, showing how slight a separation suffices to establish the complete physiological autonomy of the individual nucleus (*fig. 33*).

All of the mitoses of Synchytrium are of the same type. It is true that none of the later nuclei undergo such a shrinkage in volume with its associated peculiarities as occurs preparatory to the primary mitosis (STEVENS **18**), but these phenomena are probably due simply to the enormous size of this overgrown nucleus. It may be recalled that similar peculiarities are usually observed in very large nuclei wherever they are found. CHAMBERLAIN (**1**), for example, was not able to interpret the structures he found in the enormous egg nucleus of Dioon. But aside from these peculiarities of the primary nucleus, the only differences that could be detected were in the size of the spindles, those in the early stages being fairly large, while those in the last mitoses of the sporangium are exceedingly minute (*figs. 32–34*).

In the resting nuclei of Synchytrium the chromatin is all concentrated in a single globular karyosome (*figs. 1, 31*). Except in those cases where vacuoles have appeared on the removal of the chromatin preparatory to mitosis or nuclear gemmation (*fig. 36*), the karyosome

is an entirely irresolvable, deeply colored body, whether stained with hematoxylin or safranin. Besides the karyosome there may be a few deeply staining granules on the nuclear membrane, but there is nothing corresponding to the chromatin reticulum characteristic of the vegetative nuclei of many cells. The only nuclei in which the chromatin approached the condition of a reticulum were located in the degenerating cysts occasionally found.

The spirem

The early prophases of mitosis consist in the formation of the spirem from this compact karyosome. This is brought about in the most direct manner possible. The karyosome first separates into a number of irregular chromatin masses (*figs. 2, 30*); next delicate linin bands appear connecting these granules with the nuclear membrane (*fig. 3*); and along these bands the chromatin granules are distributed over the nuclear cavity, forming the expanded spirem (*fig. 4* from the same cyst as *fig. 2*).

Only a portion of the spirems of Synchytrium, however, are destined to pass into mitosis. A large proportion of them undergo nuclear gemmation or some other form of amitosis. In view of theoretical considerations which will be discussed later, it is of the utmost importance to determine whether the spirems of amitosis are of the same nature as those of mitosis, or whether they are different in kind and present only accidentally an optical similarity. In most cases it is easy to distinguish the two sorts by the cysts in which they occur. In those cysts which are in the prophases of mitosis, the nuclei are in general very nearly the same size and evenly spaced off from each other, while in the amitotic cysts there are small nuclei in groups or clusters as well as the large spirems. Many such spirems have a strikingly different aspect from the mitotic spirems. They are coarser (*fig. 37*), the chromatin granules are very much larger, and the linin strands are short and heavy; whereas the mitotic spirems are characterized by linin threads and chromatin granules of various sizes lying side by side. In the amitotic spirems each of the chromatin granules is evidently simply the karyosome of a small nucleus, for the formation of which the spirem is a preparation. It would thus appear that the two classes of spirems are entirely different from each other, but

unfortunately for this view a very large proportion of the amitotic spirems are intermediate, or so closely resemble the mitotic spirems that they can be distinguished from them only by the character of the cyst in which they occur (*fig. 41, a, b, c*). It seems impossible therefore to determine certainly whether these two classes are distinct or whether they are of the same nature.

The origin of the spindle

In those cells where the spindle is intranuclear, there seem to be two types of spindle formation. In the first type, which occurs in the ascomycetes (e. g., Phyllactinia, HARPER **8**) and brown algae (e. g., Fucus, YAMANOUCHI **21**), the centrosomes are permanent organs of the cell and form the spindle by moving around the nucleus so as to include the chromosomes between them, organizing the spindle by the union of those astral rays which penetrate the nucleus in a manner very similar to that prevalent among animals. This process is very conspicuous and has been observed and figured by numerous investigators. In the other type, which occurs in the oomycetes (e. g., Saprolegnia, DAVIS **3**; and Albugo, STEVENS **16**), centrosomes are either small or absent in the early stages; to this type the spindles of Synchytrium belong. Here the determination of the origin of the spindle is very difficult. This probably accounts for the fact that very few of those who have figured such mitoses have given a series of figures of the prophases complete enough to throw much light on the formation of the spindle.

When first seen, the spindle of Synchytrium, which is thrown directly across the cavity, is not distinguishable by its staining reaction or otherwise from a strand of the spirem (*figs. 6–8*). It is therefore difficult to be certain just when the spindle appears or what it comes from, but it gives every indication of being differentiated from a spirem strand. Very soon, however, it becomes sharply pointed and quite different from the spirem, which now begins to be drawn in around its equator (*figs. 9, 10*). Contraction continues and more definite connections between the chromatin and the spindle fibers are established (*figs. 11, 12*). In this condition the spindle often appears bipolar (*fig. 9*), which emphasizes the similarity of the linin strands and the spindle fibers. By further contraction this much shortened

spirem is converted into the four chromosomes of metaphase (*figs. 14, 15*). Very frequently, however, the contraction is so pronounced that the individual chromosomes cannot be made out in the chromatic mass at the equator (*fig. 13*).

Some spindles show nucleoli lying in the nuclear cavity beside the spindle; in others no nucleolus is present. No difference was observed between the spindles with nucleoli and those without; but it was observed that in any given cyst all of the spindles were alike in this respect—either all had nucleoli or all lacked them. In rare cases more than one of these bodies were present. The nucleoli remain beside the spindle till after the daughter nuclei have separated in telophase (*figs. 22, 29, 33*), when they disappear.

As soon as the spindle is formed, the nuclear membrane with the small chromatic granules which are imbedded in it begins to disappear (*figs. 10–15*), soon leaving the spindle free in the cytoplasm. As is usual with the intranuclear spindles of fungi, the metaphase in which the chromatin is all concentrated at the equator, if we may judge from the frequency with which it is observed, is of long duration. This is in contrast with the mitoses of the higher plants, where good metaphases, far from being the commonest, are observed less frequently than other stages.

Anaphase and telophase

The spherical chromosomes are pulled away from each other in the usual manner by the fibers of the spindle, which are exceedingly heavy (*figs. 15, 17*). In early anaphase, figures stained with anilin safranin and gentian violet show the chromosomes red and the spindle violet but in hematoxylin preparations the chromosomes are difficult to differentiate from the spindle fibers. This difficulty increases in late anaphase until it becomes impossible to distinguish chromatic from achromatic structures, even when stained with the safranin-violet combination. When the chromosomes pull apart, they remain connected by heavy fibers similar to those by which they were pulled away from each other (*fig. 18*), which persist long after the chromosomes become lost in the condensed mass at the poles (*fig. 19*). These deeply staining fibers are very conspicuous and give a characteristic appearance to side views of anaphases, which somewhat resem-

bles the daughter stars formed in mitoses where the chromosomes themselves are elongated. These fibers may be used to determine the chromosome number, since each one of them is connected with a chromosome. This can be done more easily in this stage than in metaphase, because the fibers are larger than the chromosomes and because they spread apart as they separate, sometimes giving views of all four at once, while in any other stage the full number can be seen only from polar view (*fig. 16*).

These radiating bands of fibers are now drawn in, leaving the two poles connected only by a central band (*figs. 20, 22*), which becomes considerably attenuated as the two chromatic masses move farther and farther apart. The daughter nuclei become separated by distances considerably greater than the original length of the spindle. In moving apart one or both of them frequently swerves from the axis of the spindle, so that the connecting fibers meet at a considerable angle (*fig. 33*). As was indicated in the paper on the reconstruction of the nucleus (GRIGGS 4), this habit makes it almost impossible to associate the daughter nuclei in pairs after the wisps of spindle which point to the original position of the equator have disappeared, especially since the wide separation greatly lessens the chances of securing both in the same section. For this reason all figures of stages after this period are drawn from only one of the daughter nuclei. After the spindles have broken in two, the telophases are very inconspicuous objects; though they stain deeply, the chromatin is concentrated into so narrow a space that they are practically invisible under any but the highest power (*figs. 23, 35b*).

The aster

As previously reported by STEVENS, KUSANO, and myself, there are no granules, radiations, condensations, or any other indications of the presence of centrosomes at the poles of the spindle during prophase or metaphase. But about the time the two halves of the spindle separate, radiations begin to emanate from the chromatic masses. At first so delicate as to be on the very limit of visibility, the aster rapidly increases in prominence until it becomes exceedingly conspicuous, clearly visible under a magnification of forty or fifty diameters. At first the radiations may appear to emanate from the

center of the chromatic mass (*figs. 24, 25*), but very soon it is evident that the focus is beyond the condensed chromosomes (*fig. 24*). As the rays increase in strength the focus is shifted, until there is a considerable interval between the center and the chromatin (*fig. 27*). In this stage, since the chromatin is condensed to its minimum volume, the aster is so very much more prominent than the chromatin that the latter is likely to be overlooked altogether, making it appear that the cyst has no nuclei, but only asters! The chromatic mass soon enlarges, however, and rounds off into the spherical karyosome of the resting nucleus (*figs. 27, 28*). Up to this stage the chromatin lies suspended in the cytoreticulum, without any apparent relation to it. The appearance of the karyosome, however, marks the resumption of definite relations of nucleus and cytoplasm in the formation of a vacuole around the chromatin (*fig. 28*). This vacuole is at first bounded only by the meshes of the cytoplasm, but soon the rays of the aster bend around it and form the heavy nuclear membrane which incloses it (*figs. 29, 30*), as previously described by KUSANO (**10**) and myself (**4**). When the nuclear membrane is complete, the aster gradually disappears; the rays first become much more numerous and finer; the center gradually becomes diffuse and stains less deeply (*fig. 4*); and finally the aster is transformed into a condensation of cytoplasm as previously described (*figs. 31, 20, 3*).

During the disappearance of the aster, however, the nucleus usually enters into the prophases of the next division, so that only seldom is a cyst found where the nuclei are still in their vegetative condition when the asters are in their last stages. On the other hand, it is not at all unusual to find that the new spindle has already formed, before the aster has disappeared, and in rare cases the anaphase of the succeeding division (*fig. 20*) may be reached before the old aster is completely gone. The disappearance of the aster probably occupies a period of somewhat definite length, while the rapidity with which the mitoses succeed each other varies greatly in different cysts. It was this lack of correspondence between the cycles of astral and nuclear metamorphoses that made it necessary in the former paper on this subject to state some conclusions provisionally that may now be positively established.

From their function of forming the nuclear membrane, the asters

of Synchytrium have been named *karyodermatoplasts* by KUSANO (13). Although somewhat long, this term may be useful in alluding to the *function* of the asters, but there seem to be certain objections to its use as the name of a structure. First, the structure so designated is so variable that it is difficult to define it. Sometimes the aster is single, sometimes double or triple (*fig. 2*); sometimes it has one clearly defined granule at the focus of the rays; more often there are several such granules more or less eccentrically placed; or there may be none at all. Sometimes there is an elongated band which bears radiations all along its length, like the blepharoplast of a cycad. It is evident that the term karyodermatoplast can be defined only by its function, while such a term should rest on a morphological basis. Second, though the aster, so far as observation has yet indicated, serves only to reconstruct the nuclear membrane, that function alone does not seem to the writer adequate to account for its enormous development in Synchytrium. This is more apparent when one recalls the fact that in heteroschizis and nuclear gemmation the membranes of the daughter nuclei are formed without the intervention of any such structure. I have preferred, therefore, in the present discussion to employ the descriptive term aster for the structure in question, without committing myself to its significance. The question of the radiate structures in Synchytrium and their homology is one of very great interest and deserves consideration in a separate paper.

The chromosome number

Inasmuch as most of the nuclei or their ancestors have been derived by amitosis, as has been previously shown, the determination of the number of chromosomes in Synchytrium becomes a matter of much more importance than in organisms where the orderly sequence of mitosis has not been interrupted. On this account especial care has been taken to insure accuracy of observation and interpretation. Upward of 500 slides have been used in the study. Altogether several hundred mitotic cysts with many thousands of individual spindles have been observed. Of these only the most favorable were used for basing the conclusions. The location of the cysts containing these most favorable spindles was recorded by vernier readings of the mechanical stage and filed in a card catalogue, so that they could be

reexamined in rapid succession as frequently as desired. The favorable cysts so listed number about fifty; most of them contain several hundred spindles.

All of the results obtained were not exactly concordant, but I shall give the observations on which the conclusions were based that the reader may be enabled to judge of their soundness for himself. In all but two of these favorable cysts there were constantly four chromosomes. In these two, however, the number of chromatin bodies was certainly more than four (*fig. 42*). But while the chromosomes are all of the same size, some of these bodies were smaller than chromosomes and had the appearance of masses of chromatin from the late spirem which had not yet fused together into the compact chromosomes of metaphase. Inasmuch as the spindles were undoubtedly newly formed, this is probably the correct interpretation, especially as there seemed to be in some cases faint wisps of spirem remaining about the equator of the spindle. But whether these supernumerary chromatin bodies are to be explained on some such basis or whether they are actual irregularities in the number of chromosomes, I am of the opinion that they do not seriously weaken the conclusion that the chromosome number is four. Although it is unusual for a writer to state difficulties of this sort as frankly as has WILSON (**20**) in his study of Metapodius, seeming discrepancies in the number arising from one cause or another are, I believe, frequently met with in efforts to count chromosomes.

In all of the other cysts studied there was no deviation from the constant number four. On all of the spindles the chromosomes were placed at angles of about 90°, so that two, three, or four of them were visible, according to the angle of observation (*figs. 14–19*). In the very much larger number of cysts whose spindles were not favorable enough to permit exact counting, there were no indications of a different number. It should be noted also that four is an exceedingly easy number to count, for one can *see* four without counting them one by one as is necessary for a larger number. In thus maintaining a constant number of chromosomes the writer is supported by the only other published accounts of the chromosomes of Synchytrium. STEVENS in his paper on the primary mitosis (**18**) states (p. 413) that "they are probably four in number, although we do not assert

this with certainty," and in his second paper he shows spindles with the same number of chromosomes. KUSANO (10) reports definitely five chromosomes in *S. puerariae*.

The probability that the results thus obtained are accurate is very greatly increased by the fact that in Synchytrium there is no differentiation into soma and germ plasm. Every nucleus becomes either directly or through its descendants the nucleus of a spore, which, if successful in entering its host, becomes the large nucleus of a primary cyst. If a variation of the chromosome number occurred at any point, it would, on the individuality hypothesis, be perpetuated indefinitely, affecting all the nuclei of later generations; whereas in the higher plants and animals a variation in the somatic nuclei would be lost with the death of the individual organism in which it occurred. It is clear, therefore, that if a variation in the number of chromosomes should occur only once in the repeated direct divisions through which the nuclei of the spores have been derived, it would affect all the nuclei of the next generation. Thus, though the nuclei of the later stages of the parasite are so small that an irregularity in the chromosome number might not be detected, yet in the large nuclei of the succeeding generation it could not escape observation. Amitosis plays so important a part in the formation of the nuclei that if chromosome variations occurred in only one per cent. of the direct divisions, the nuclei of the whole parasite would in the course of a few generations become so irregular that it would be impossible to recognize the original number of chromosomes when it did occur. It may also be remarked that an irregular reduction in the number of chromosomes, such as might be expected in the amitoses of a non-sexual organism like Synchytrium, could lead, when repeated in indefinite series, to only one result—all the nuclei would finally have only one chromosome.

On the individuality of the chromosomes

Modern cytology may be almost said to be built around the theory of the individuality of the chromosomes, and certainly no other hypothesis has borne so much fruit in valuable results as this. Without it the complicated process of mitosis would seem to lack significance, and the doubling and reducing of the chromosomes in

fertilization and reduction would be well-nigh meaningless. For its support it has had, besides the constancy of the chromosomes in the species, various observations on nuclei which were known to contain either more or less than the normal number of chromosomes.

The great difficulty in the way of the theory has always been that the chromosomes apparently lose their individual existence during the vegetative phases of the nucleus. In many cases where the nuclei are rapidly dividing the chromosomes do not, however, entirely lose their individuality between the successive divisions, but divide and reproduce themselves directly from the compact condition of mitosis. These cases have been the evidence by which the vegetative period of the nucleus was bridged over by the individuality hypothesis, for there is an unbroken series of intergradations from this condition to that in which the chromosomes have completely lost their morphological identity, and one would not suppose that nuclei in one condition differed fundamentally from those in the other. Accordingly, basing their statements on observations of nuclei in which the individual chromosomes could be traced, with more or less certainty, from mitosis to mitosis, several writers have asserted confidently that the individuality of the chromosomes is maintained through all conditions of the chromatin, whether visible or not.

But the transformations of a set of chromosomes which, after they are once formed, persist and divide are not necessarily equivalent to the formation of a new set from a diffuse reticulum. If the chromatin reticulum gave rise only to the chromosomes, the analogy might be closer; but when, as frequently happens, large amounts of chromatin are cast out into the cytoplasm as nucleoli, microsomes, or in mass, it is evident that the succeeding differentiation of the chromosomes involves factors different from those entering into the mere transformation of a chromosome which persists *intact* through whatever metamorphoses it may pass. This may be illustrated perhaps by comparing the chromosomes to metal rods which remain distinct from each other when cold, but as the temperature approaches the melting point become soft, lose their shape, and partially fuse together, though each retains its distinctness and can be reclaimed unchanged on cooling. But after they have once melted together, all this is changed, and they can be regained from the homogeneous flux only by

making them entirely over again. It seems quite possible that in the diffuse vegetative reticulum the chromosomes are as completely fused as the molten metal, and until we know more than we do now concerning the nature of the reticulum, this alternative possibility should be kept before us. The analogy of the molten metal is more suggestive in cases like Synchytrium, where the chromatin is partially or completely concentrated in a karyosome from which part or all of the spirem is directly derived. Although cytologists have as a rule paid but little attention to their behavior in mitosis (cf. WAGER 19), such structures are common occurrences in many plants and animals.

If we may infer safely, as I believe we may, that the chromosomes of *Synchytrium decipiens* number constantly four, it becomes a matter of primary interest to determine how that constant number is maintained in all of the amitoses through which the nuclei pass. It has long been assumed that the principal if not indeed the sole function of the complicated process of mitosis was to insure exactly equal division of the chromatin between the daughter nuclei. In view of the fact, however, that the majority of the nuclei of Synchytrium are derived by some form of amitosis either directly or through their ancestors, and yet maintain the number of their chromosomes constant, it is evident that mitosis is not necessary to maintain this number.

The possibilities of an exact mechanical division of the chromatin are somewhat different in the different varieties of amitosis. In heteroschizis there occurs a metamorphosis of the nucleus suggestive of that of mitosis. In the loss of the nuclear membrane and the apparent cessation of interaction between the nucleus and the cytoplasm there may be a pause during which the chromatin is divided granule by granule in such a way that the karyosomes of the daughter nuclei are furnished with exactly equal chromatin content, just as is visibly accomplished by the fission of the chromatin granules or the chromosomes in mitosis.

Likewise it is not inconceivable that the karyosome may be divided in a similar manner when it is broken up preparatory to nuclear gemmation, though in this variety of amitosis there is no loss of the nuclear membrane or other indication of a pause in metabolism.

An exact equational division of the chromatin under such circum-

stances would be something of a novelty, but it would not necessarily involve functions of the chromatin differing fundamentally from some with which we are more familiar. Whenever the chromatin is withdrawn granule by granule from a karyosome (nucleolus) preparatory to mitosis, we must suppose, on the individuality hypothesis, that the granules come out of the nucleus as they went in, properly sorted, so that each granule is taken into that chromosome from which it came. On the assumption of an equational division in heteroschizis and nuclear gemmation, we should have to assume, in addition to this well-known property of the chromatin granules to sort themselves out from the apparently homogeneous mass of chromatin of the karyosome, only the further property of reproducing themselves while yet within the karyosome as they ordinarily do in the spirem. This is perhaps not too much to ascribe to the chromatin, but even such an addition to our theory would add a very large field for speculation where the conclusions, with our present methods, could never be checked by observation.

But when nuclear gemmation takes place in spirem two alternative possibilities are presented, the choice between which depends on the nature of the spirem involved. If the spirem is an entirely different structure from the mitotic spirem, it would be rational to suppose that each small karyosome, i. e., each granule of the spirem in nuclei such as *fig. 37*, contains four chromosomes derived by an equational division of the mother karyosome as in the previous cases. But if the amitotic spirem is like the mitotic, the situation is entirely different, for we know from its later history the composition of the mitotic spirem. It contains altogether only four chromosomes, which are arranged serially, and any small part will contain chromatin from only *one* chromosome, if a small part of such a spirem is extruded and forms an independent nucleus. Therefore, it is evident that its chromatin is derived not from all four but from only one chromosome. It is then a matter of great importance to determine the constitution of the spirem, but unfortunately, as stated above, the evidence on this point is somewhat ambiguous, and a definite decision in favor of either alternative seems impossible.

Certain features of nuclei dividing by constriction, however, seem to throw some light on this matter. When amitosis by constriction

occurs, the nucleus develops lobes and divides up into a number of daughter nuclei varying from two to a dozen or even more (*fig. 41, a, c*). One cannot tell by observation whether a nucleus is about to divide into two or many daughters, for there is no apparent segregation of chromatin into parts corresponding in number with the number of small nuclei to be formed. Nor are the small nuclei necessarily equal in size or chromatin content. Sometimes the variations are very great; thus *fig. 36* shows a cyst in which the primary nucleus has divided, leaving the nucleolus undivided in one of the daughters, while the other and smaller daughter nucleus has constricted or budded again to form a relatively minute nucleus at one side. It is difficult to believe that these three nuclei had their origin in equational divisions of the chromatin previous to the actual constriction of the mother nuclei. All appearances go to indicate rather that the factors controlling the division of these nuclei are entirely disconnected from the behavior of the chromatin, and favor CHILD'S hypothesis (**2**) that amitosis is merely a physical process. But whether this be the case or not, nuclei so formed are normal and sometimes pass into mitosis. When this happens they show the four chromosomes characteristic of the species (*fig. 35, a*). More commonly, however, amitosis by constriction is but an incident in the division of the chromatin, for the constricted nuclei are soon completely converted into groups of small nuclei by gemmation (see **6,** *fig. 3*). Since the constitution of these nuclei is of great importance in this connection, I have introduced here a series of three drawings of nuclei from the same cyst. *Fig. 38* shows a case of amitosis of the type most commonly observed, though it is not common in Synchytrium; in *fig. 39* the two nuclei are completely separated but still touch each other; in *fig. 40* one of the granules similar to those on the periphery of the other nuclei has formed a small nucleus by gemmation. The individuality hypothesis leads us to rather startling conclusions regarding the constitution of these nuclei. Each of the large nuclei must have four chromosomes. And since each of the granules on the periphery has the power of organizing a new small nucleus with four chromosomes, it also, if the chromosomes have a material continuity from generation to generation, must contain four chromosomes. In other words, the large nuclei must at one and the same time have four and $n \times 4$ chromo-

somes. In these nuclei the small karyosomes number 4, 5, 6, 7. The respective nuclei must therefore have 16, 20, 24, 28 chromosomes, and still have no more than four!

There seems to be no option but to conclude that in Synchytrium there is no morphological continuity of the individual chromosomes, nor is there any definite set of chromatin granules of whatever size which are passed on intact from nucleus to nucleus. The number of chromosomes seems to be a physiological rather than a morphological constant. Not the chromosome but the nucleus itself seems to be the morphological unit of the lowest order. Apparently any mass of chromatin that is capable of organizing a nucleus at all, i. e., any particle of nuclear matter that is able to continue life and reproduce itself, whether it originally contained portions of all of the chromosomes or of only one of them, will preserve the characteristic number of chromosomes along with the other hereditary characters of the species.

Although such a hypothesis seems to be required to explain the phenomena of nuclear division in Synchytrium, it would not be wise to attempt to reach any decision as to its general applicability at this time. In proposing it to account for these features of Synchytrium, the writer is not at all oblivious of the enormous amount of evidence which has been heaped up in recent years in support of the individuality of the chromosomes. The occurrence of protochromosomes in the nuclei of many cells (OVERTON **14**); the persistence of supernumerary chromosomes in cases of polyspermy, etc.; and the remarkable size and shape differences among the individual chromosomes such as have been discovered in animals, notably by MCCLUNG and MONTGOMERY, and recently in plants by SCHAFFNER (**15**) and Miss HYDE (**9**), along with many other observations of a similar nature, are facts which give very great weight to the hypothesis of the individuality of the chromosomes. Nevertheless, it must be remembered that almost all of the evidence in favor of the individuality hypothesis is of suggestive rather than demonstrative value. This was clearly recognized by all observers until about five years ago, but since that time the amount of such evidence has increased to such an extent that cytologists have been much less cautious than before in using the hypothesis. The renaissance of MENDEL'S law so favored the general

acceptance of the chromosome hypothesis by making it highly desirable on theoretical grounds to find the material bearers of hereditary units which the law seemed to demand, that practically all opposition to it was swept away, and it has come to be the groundwork on which present-day cytology has been constructed. If then the basis on which the theory rests is in any degree unproven, it is well for cytologists to keep that fact clearly before them, that they may be guarded against making any false steps in blind adherence to an insecure hypothesis or of failing to notice any evidence which might throw doubt on the validity of their theory.

The writer for his own part, however, has no intention of discarding the whole hypothesis of chromosome individuality on the basis of these observations on Synchytrium, or on the similar results obtained by CHILD (**2**) from studies of representatives of nearly all the great animal phyla. The conflict of evidence for and against the hypothesis seems clearly to require us to keep the case open for the present. It is as tending to promote such a judicial attitude of suspended judgment among cytologists rather than as overthrowing the whole theory that the writer would have his results received.

Summary

The mitoses are all of the same type and occur simultaneously throughout the cyst.

The spirems of amitosis are frequently indistinguishable from those of mitosis.

The spindle is of the oomycete type, without centrosomes.

The asters first appear as emanations from the condensed chromatic mass of telophase, but quickly separate from the chromatin and their rays form the nuclear membrane.

The number of chromosomes is constantly four.

In some sorts of amitosis an equational division of the chromatin previous to the division of the nucleus is possible, though there is no evidence of such a process.

In other varieties of amitosis an equational division of the chromatin does not seem to be possible, but rather direct division gives some indication of being merely a physical process as suggested by CHILD.

Nevertheless, nuclei known to be derived by amitosis show four chromosomes.

It is therefore concluded that in Synchytrium there is no morphological or material continuity of the chromosomes from generation to generation of nuclei; but that the chromosome number is a physiological constant, like the other hereditary characters of the species.

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Addendum

After the foregoing paper had been completed and submitted for publication, KUSANO's "Contribution to the cytology of Synchytrium and its hosts" (Bull. Col. Agr. Imp. Univ. Toyko **8**:80–147. *pls. 8–11*. 1909) reached me. KUSANO's paper, for the most part, is based on observations of *S. puerariae*, which seems to be remarkably similar to *S. decipiens*, which he also used for comparison. He gives much space to the metamorphosis of the nucleolus (karyosome), which is unusually favorable for study in Synchytrium, showing that all the elements derived from the mother nucleus are concentrated in the karyosome of the daughter nucleus from which they are later withdrawn. He figures the prophases and metaphases of the primary mitosis, confirming for the most part STEVENS' observations, but like him failing to find the anaphases and telophases, which for some reason seem to be very difficult to observe. He then devotes considerable space to the secondary mitoses, obtaining results similar to those of the present paper; but his account of the prophases differs considerably from that of the present writer. His series of figures, however, is not complete at this stage, and he admits (p. 102) that he had not been able to follow the formation of either the chromosomes or the spindle. Again, in the telophase there is a wide gap between his *figs. 55* and *56*, during which the aster is developed, a process about which he was left to conjecture (p. 127), incorrectly supposing that it originates from the cytoplasm. In addition to the method of segmentation by cleavage furrows described by HARPER, he reports a second method in which the sporangium walls are precipitated by the cytoplasm as in the endosperm of the higher plants. This form of segmentation occurs also in *S. decipiens* and, according to my observation, is more common than that described by HARPER.

KUSANO saw and figured the clusters of small nuclei due to various sorts of amitosis as described in my former paper (5), but he did not study them carefully and failed to ascribe to them the importance that they really possess. He repeats the statement of his preliminary paper that the chromosomes number constantly five, and thus adds strongly to the case against the individuality of the chromosomes made in the present paper.

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EXPLANATION OF PLATES XVI–XVIII

All the figures were made with a 1.5^{mm} immersion objective and ocular 12 giving a magnification of 2130, excepting *fig. 36*, which was drawn under a 4^{mm} dry lens with a magnification of 400. They were reduced $\frac{1}{3}$ in reproduction, canceling the enlargement due to the camera and rendering them the same size as they were seen in the microscope.

PLATE XVI

FIG. 1.—A vegetative nucleus.

FIGS. 2–4.—Three stages in the formation of the spirem from the same cyst, showing also the asters of the preceding division in process of disintegration.

FIG. 5.—A large nucleus in spirem beside which is a small nucleus, derived by gemmation, still in the vegetative condition; from the same cyst as *figs. 6–9* and *13*.

FIGS. 6–9.—The development of the spindle, from the same cyst.

FIGS. 10–12.—The contraction of the spirem around the equator of the spindle; nuclear membrane with its chromatic granules dissolving; from the same cyst.

FIG. 13.—A young spindle with the chromatin so densely aggregated around the equator that different parts cannot be made out; from the same cyst as *figs. 5–9*.

FIG. 14.—A spindle at metaphase, showing three of the four chromosomes.

FIG. 15.—Chromosomes enlarging as they divide, each of them attached to the pole by heavy fibers.

FIG. 16.—A polar view of metaphase, showing the four chromosomes and the nucleolus.

FIG. 17.—Chromosomes divided and beginning to separate.

FIG. 18.—Anaphase, showing separating chromosomes and the heavy fibers which connect them across the equator of the spindle; from the same cyst as *figs. 15–17*.

FIG. 19.—Anaphase; chromosomes lost in the deeply staining mass at the poles; connecting fibers prominent.

PLATE XVII

FIG. 20.—Anaphase similar to *fig. 19*, but remarkable for the very late persistence of the aster from the previous division.

FIG. 21.—Similar anaphase, with the aster appearing unusually early.

FIG. 22.—Connecting fibers withdrawn.

FIG. 23.—Spindle breaking apart in the middle.

FIG. 24.—Radiations from the chromatic mass just beginning to appear at the lower pole; at the upper pole the focus has shifted out beyond the chromatin.

FIG. 25.—A stage in the development of the aster intermediate between the two poles of the spindle shown in *fig. 24*.

FIG. 26.—Aster strongly developed, while the chromatin remains extremely closely condensed; from the same cyst as *fig. 24*.

FIG. 27.—Chromatin beginning to enlarge to form karyosome.

FIG. 28.—Karyosome rounded off; nuclear vacuole just appearing.

FIG. 29.—Rays of aster beginning to bend around the vacuole to form nuclear membrane; nucleolus and remnant of spindle lying below daughter nucleus.

FIG. 30.—Rays of aster nearly surrounding the nuclear cavity; karyosome passing into spirem of the next mitosis; cf. *fig. 2*.

FIG. 31.—A vegetative nucleus with the old aster lying along side.

PLATE XVIII

FIG. 32.—Spindle of a primary nucleus with halo and disintegrating nucleolus.

FIG. 33.—Two sporangia from a newly segmented summer sorus, showing the character of the mitoses and the independence of the segments.

FIG. 34.—A spindle of one of the last mitoses of the sporangia.

FIG. 35.—*a*, group of spindles formed by the division of a cluster of nuclei formed by amitosis, which lies in the center of a large cyst, the periphery of which contains the spindles of many small nuclei (*b*) derived by gemmation.

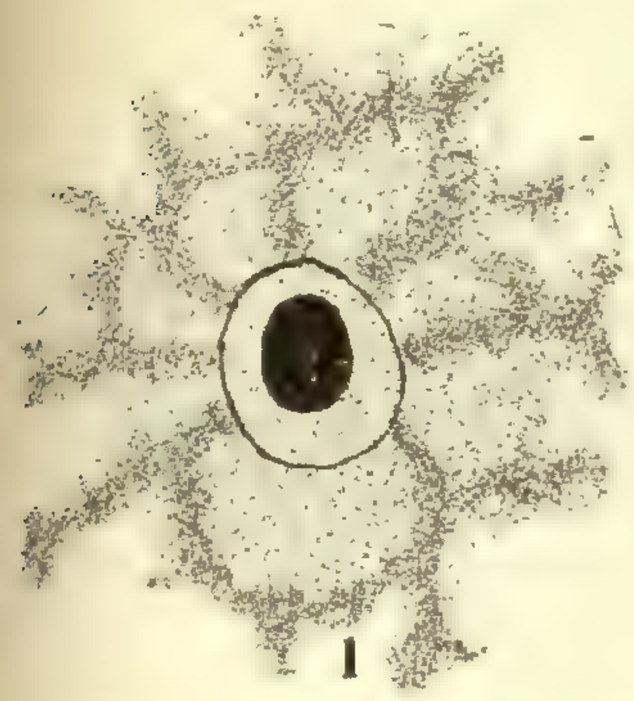
FIG. 36.—Amitosis by constriction in a primary cyst, showing very great differences in chromatin content of daughter nuclei.

FIG. 37.—An amitotic spirem in preparation for nuclear gemmation.

FIGS. 38–40.—Amitosis by constriction and by nuclear gemmation in the same cyst.

FIG. 41.—*a*, two amitotic nuclei; *b*, an adjacent nucleus in spirem, which though undoubtedly amitotic resembles mitotic spirems of *figs. 4, 6; c*, a cluster of amitotic nuclei from the same section.

FIG. 42.—A spindle with five chromatin bodies, presumably chromatin granules of the spirem not yet fused into chromosomes; cf. *fig. 11*.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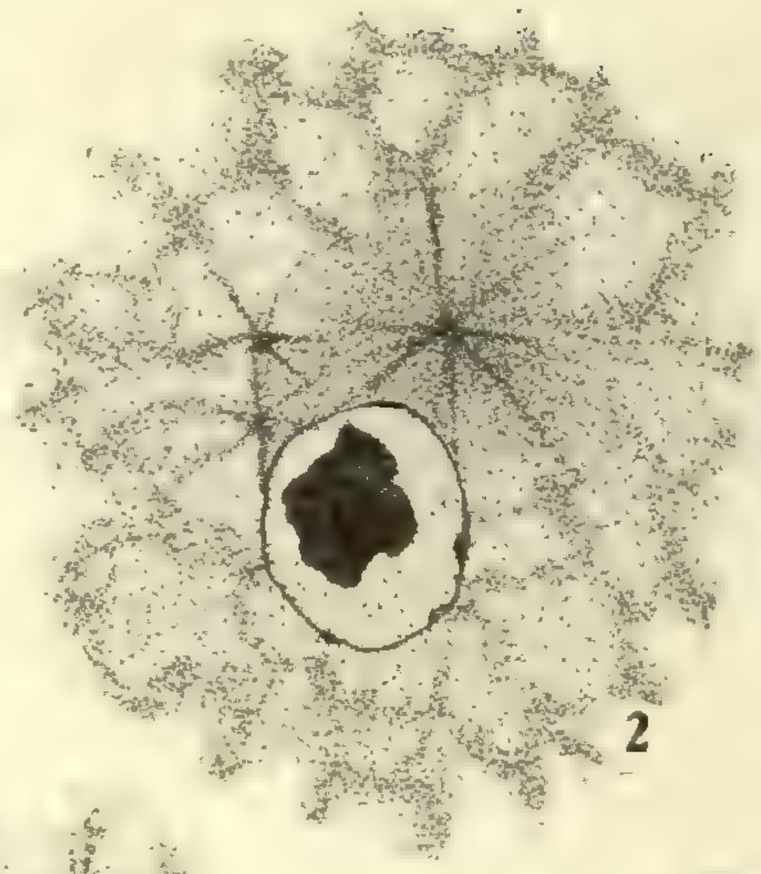
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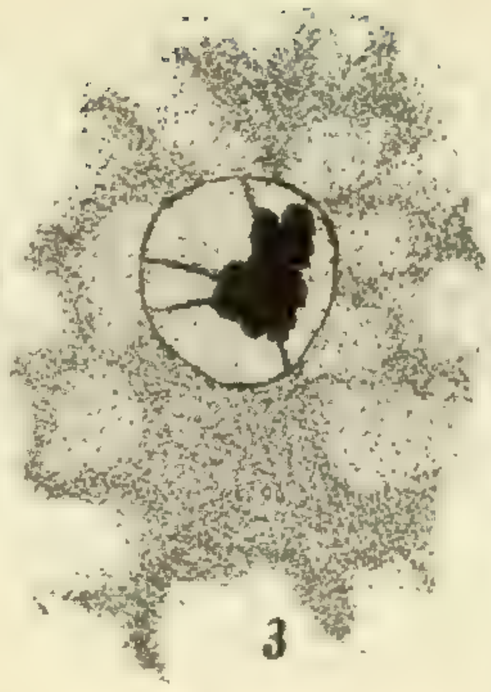
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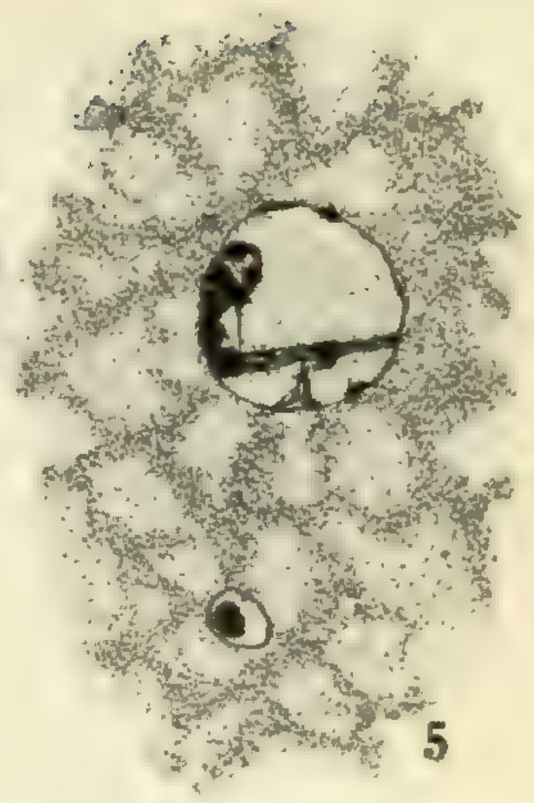
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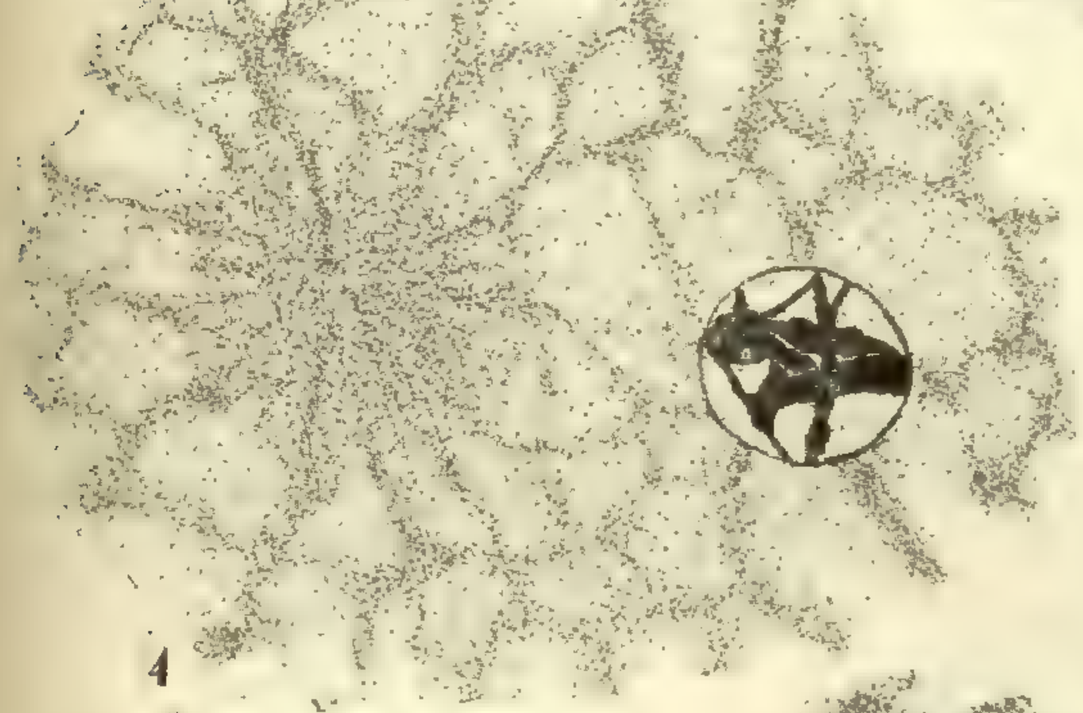
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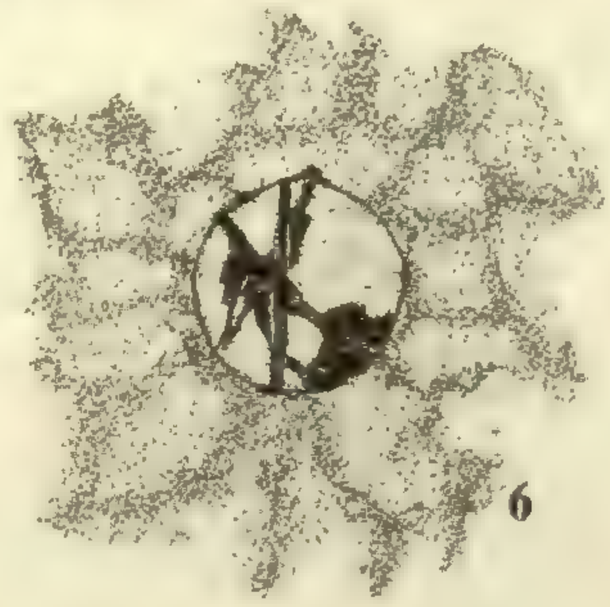
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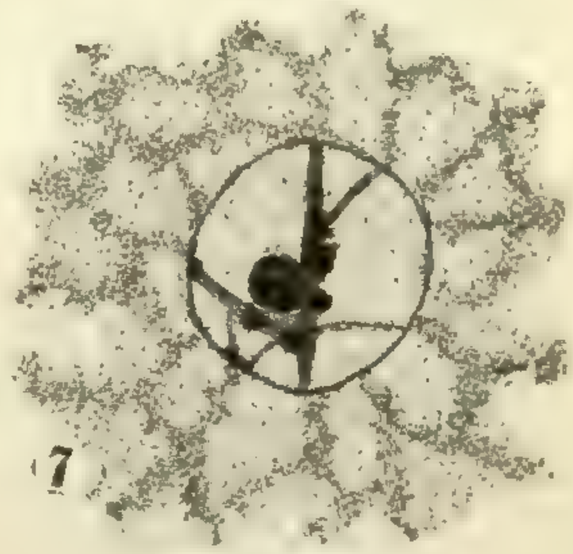
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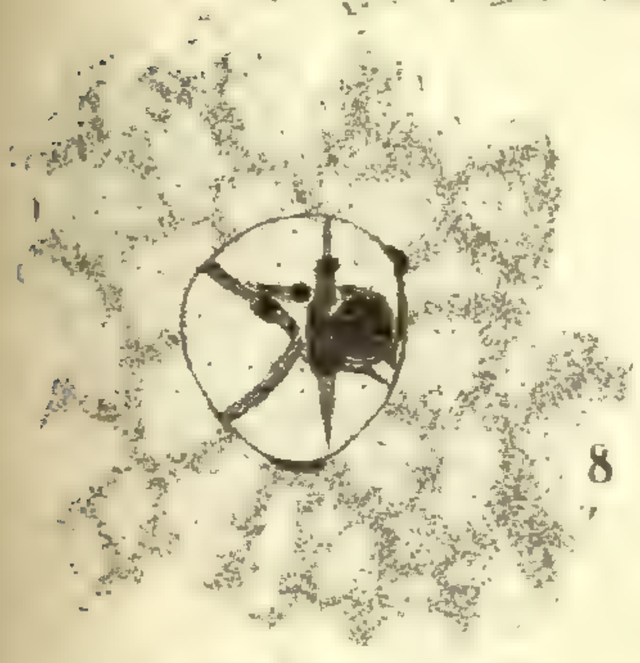
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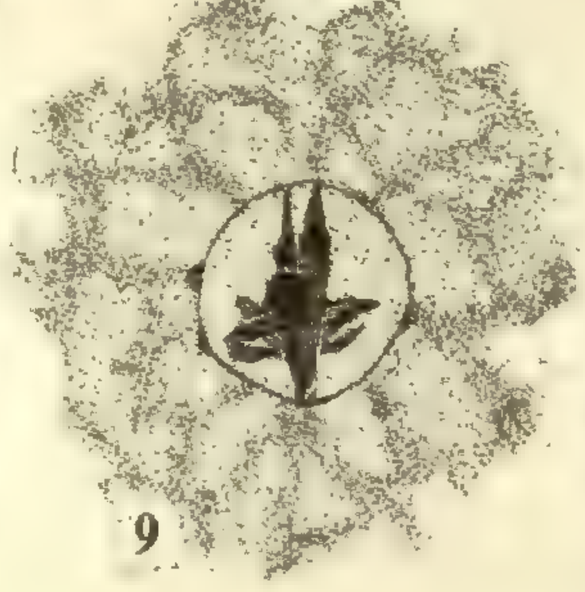
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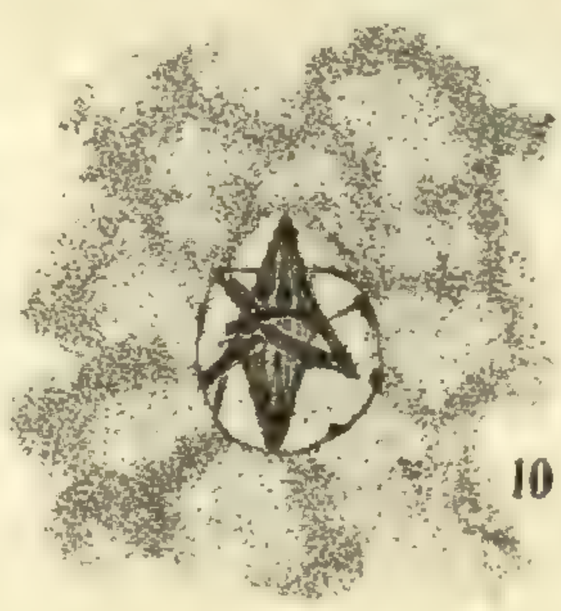
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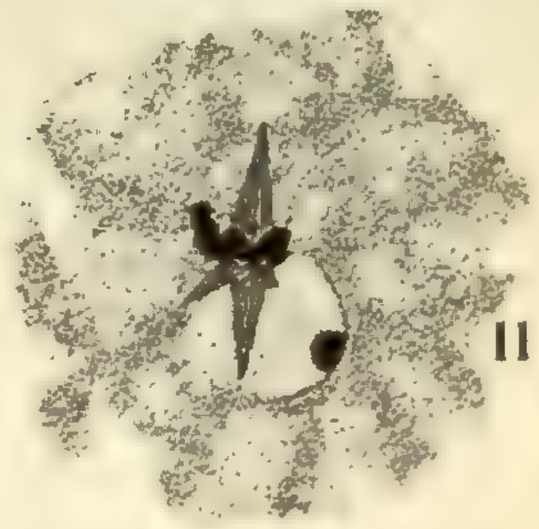
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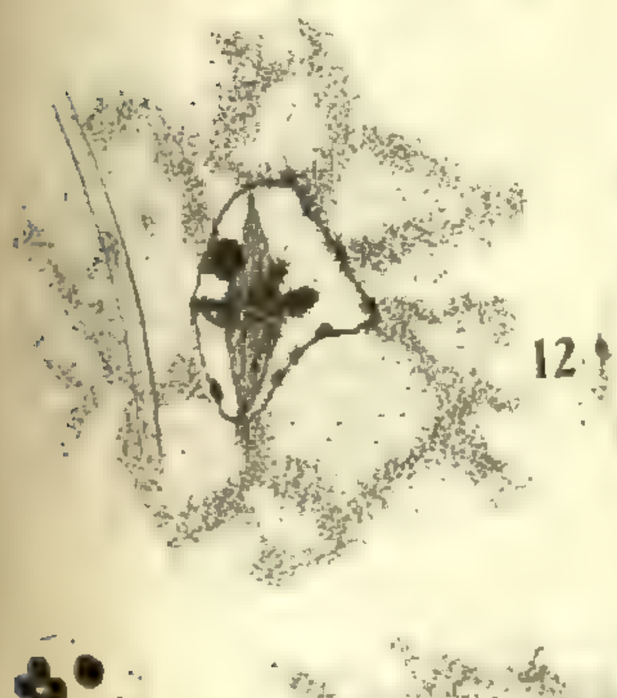
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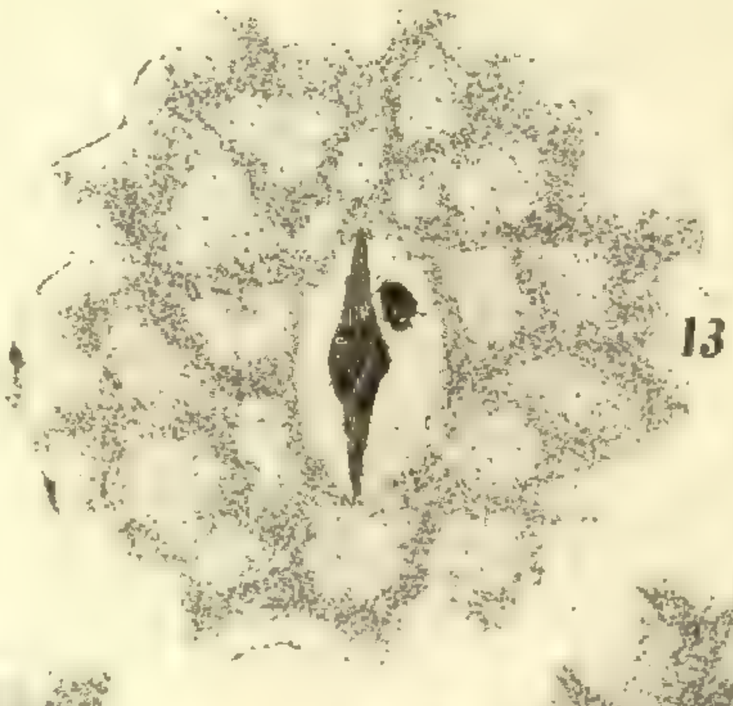
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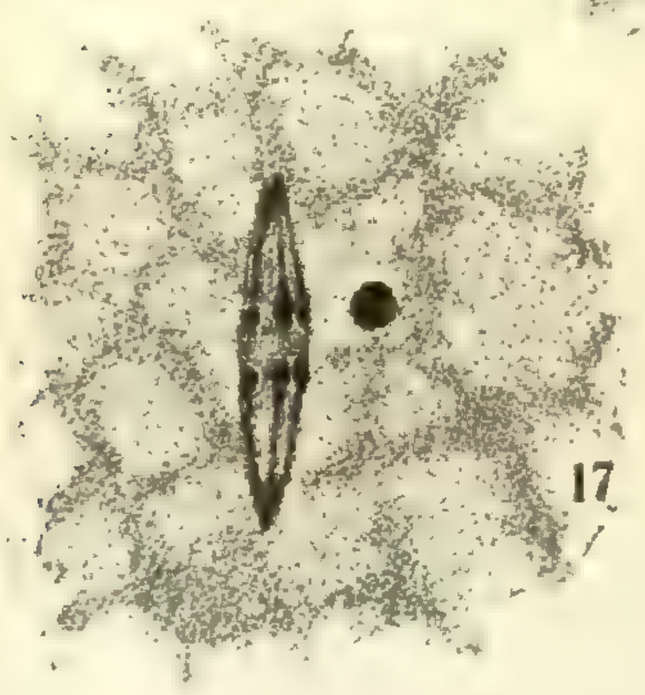
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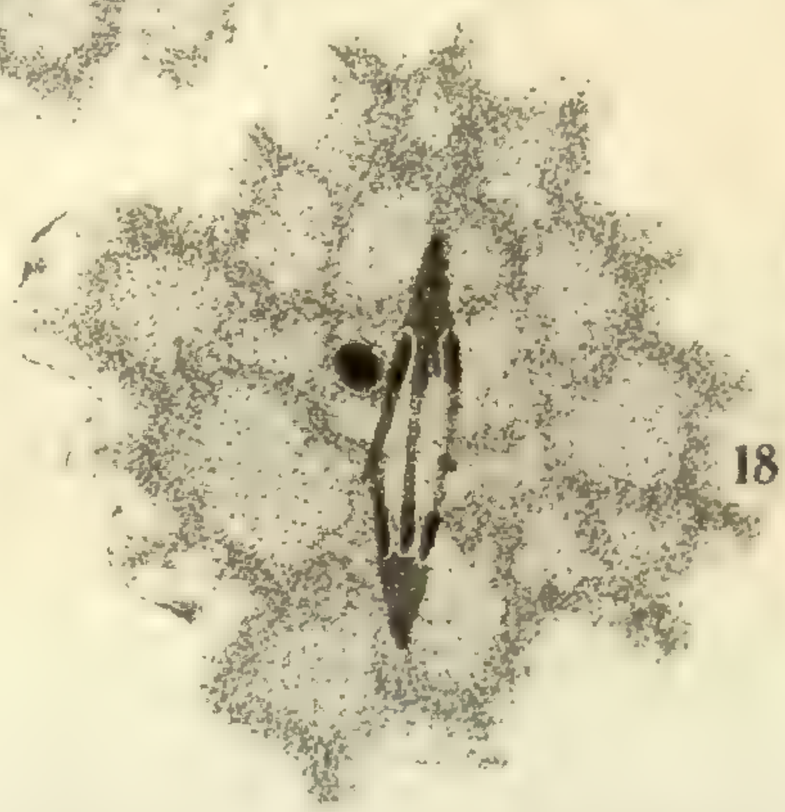
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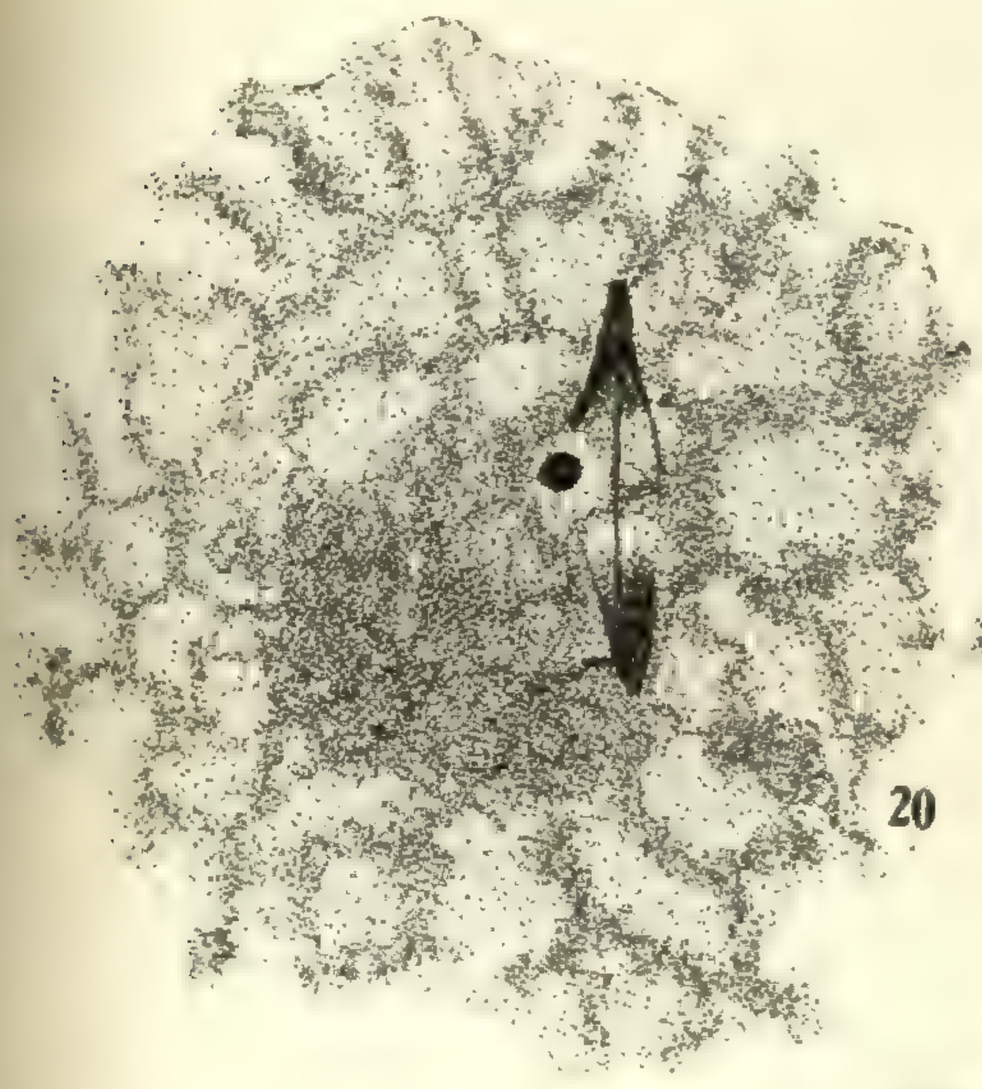
GRIGGS on SYNCHYTRIUM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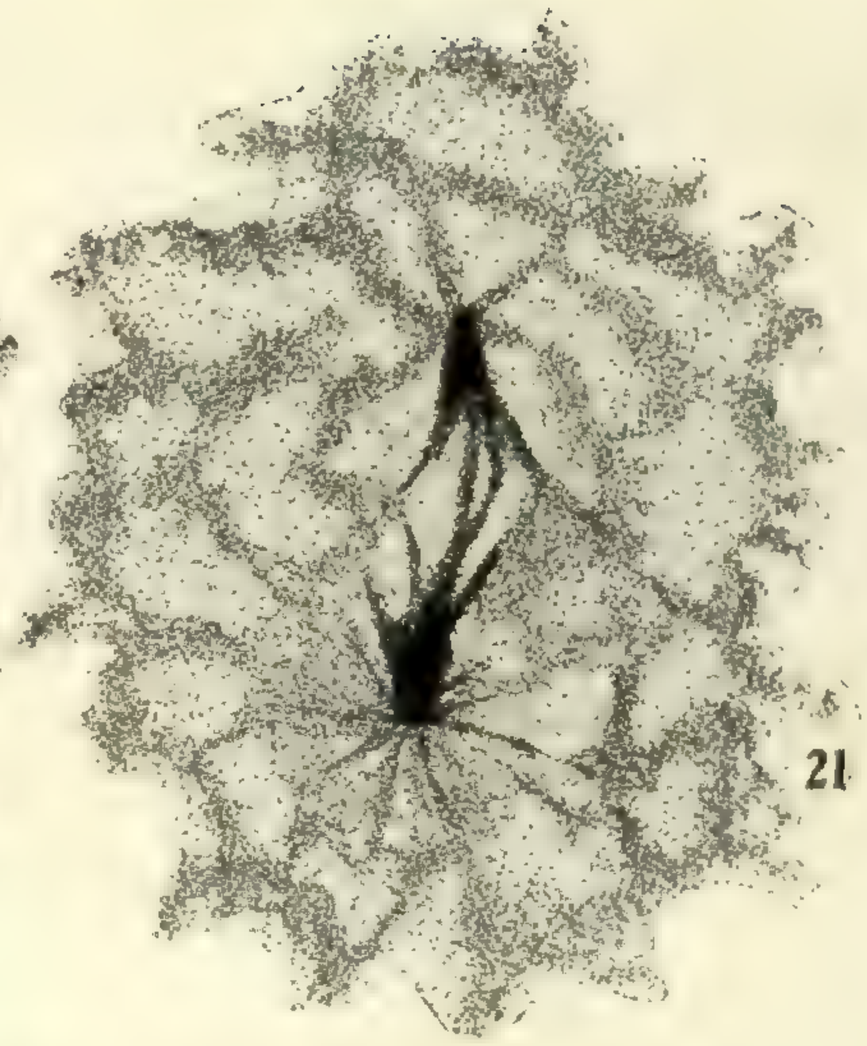
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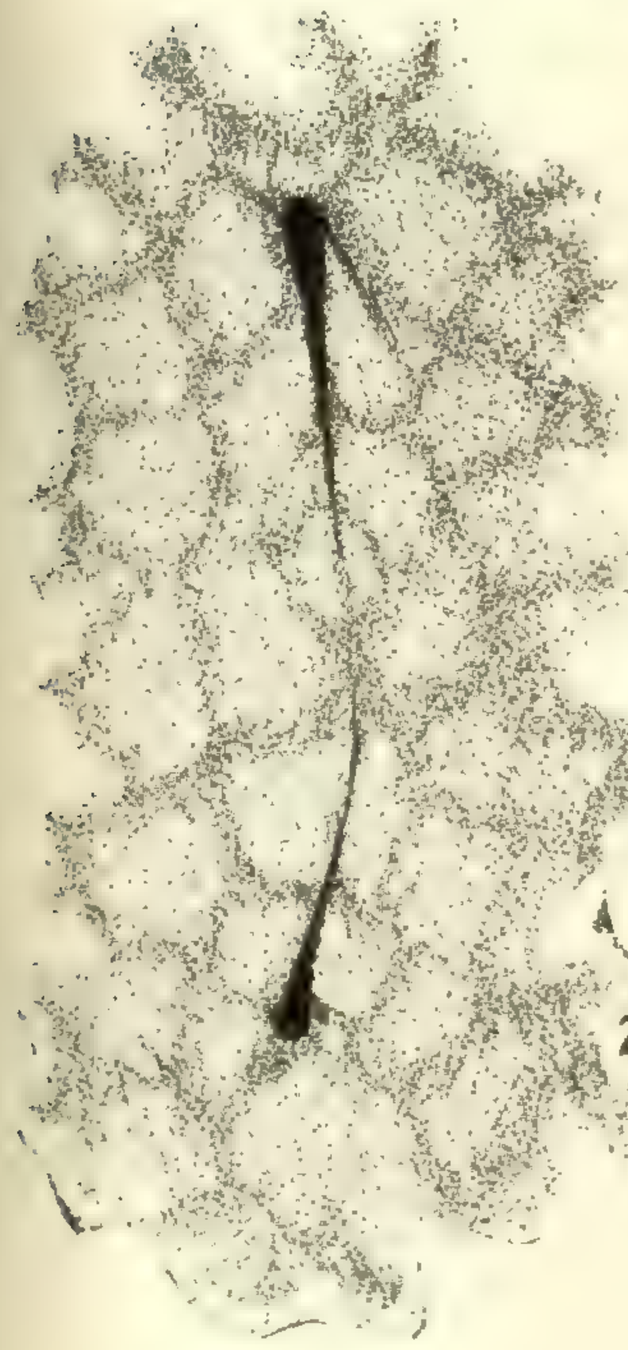
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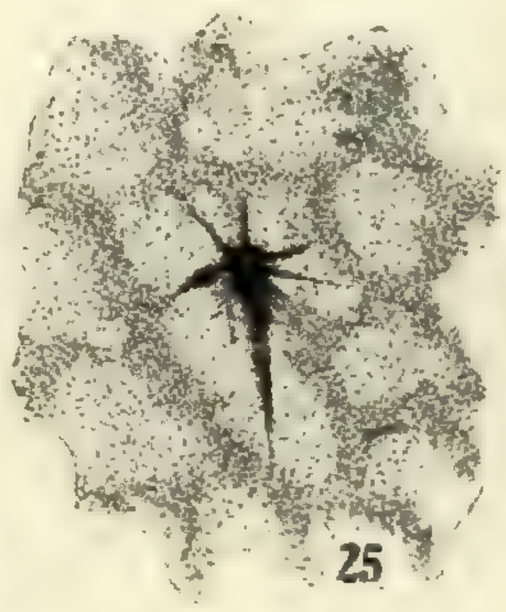
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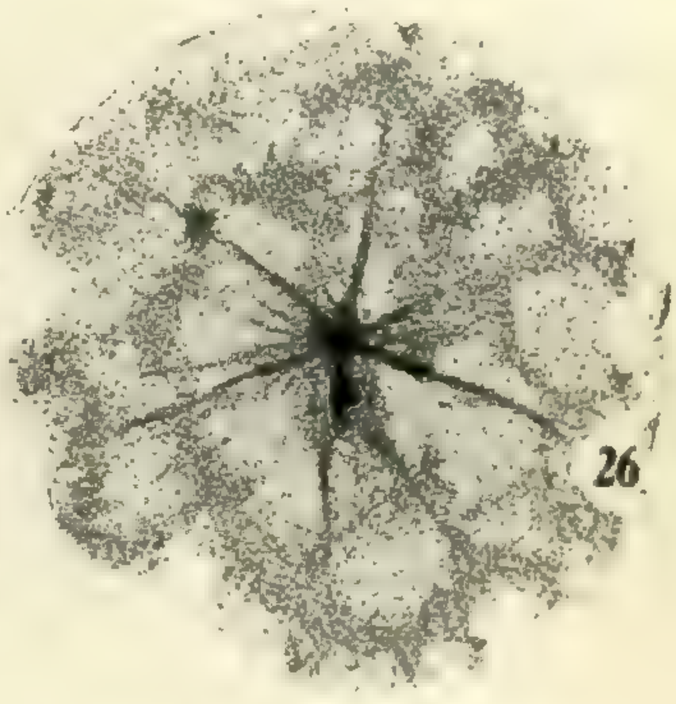
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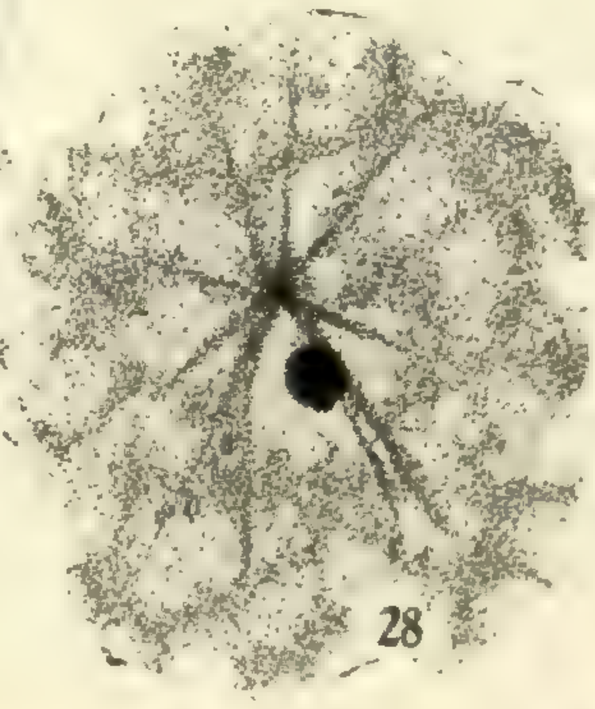
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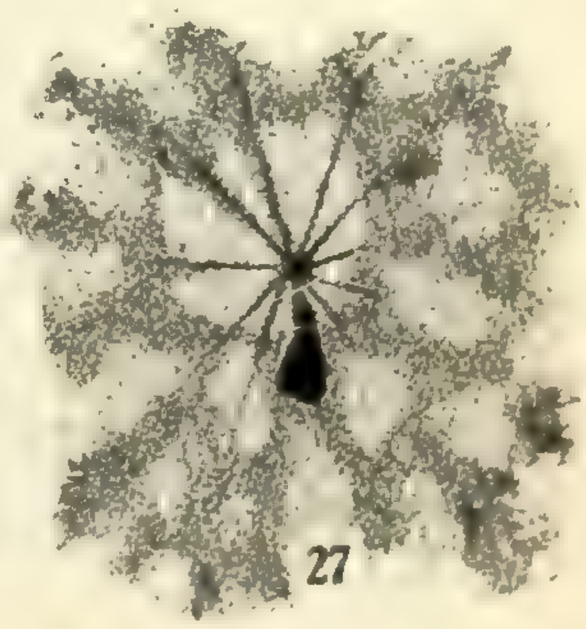
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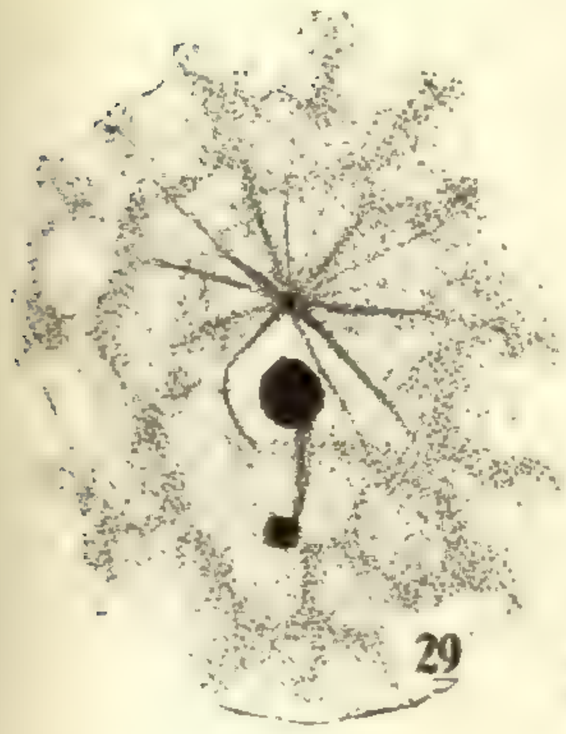
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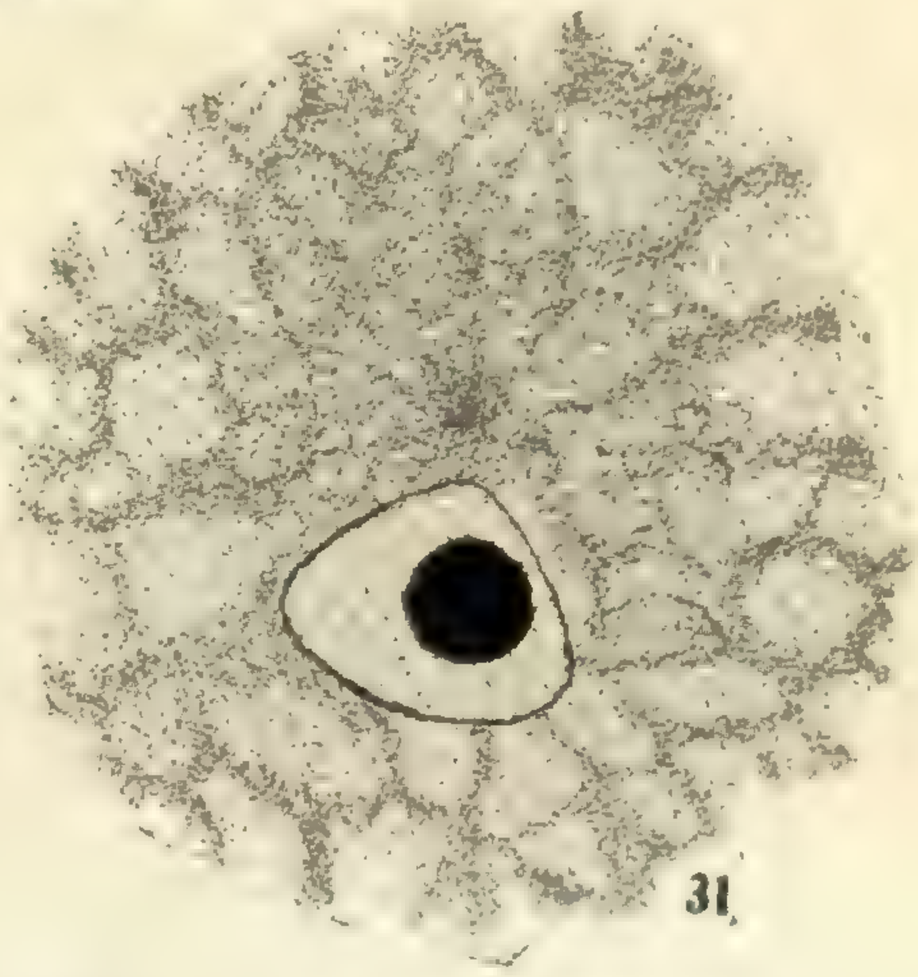
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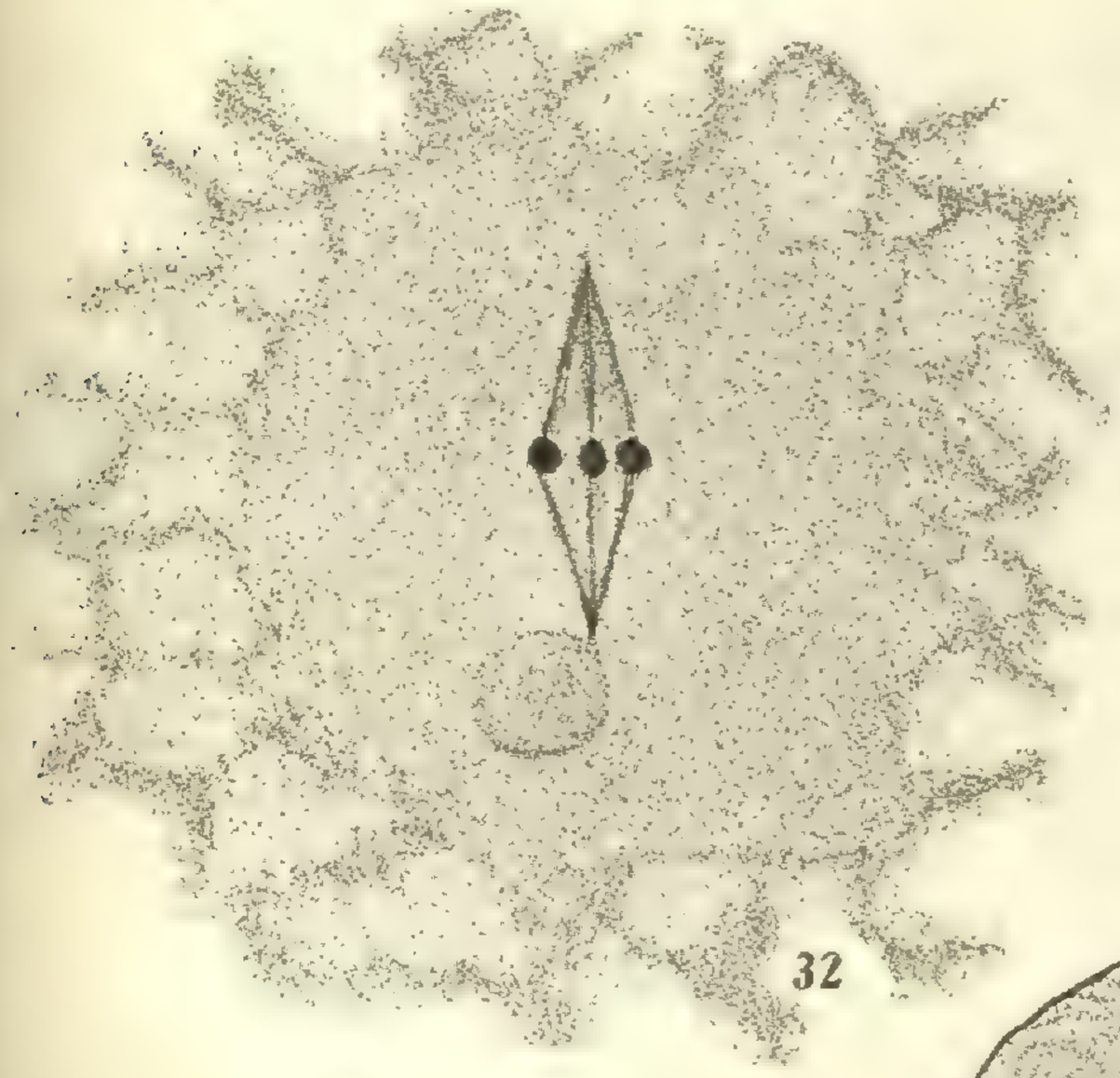
GRIGGS on SYNCHYTRIUM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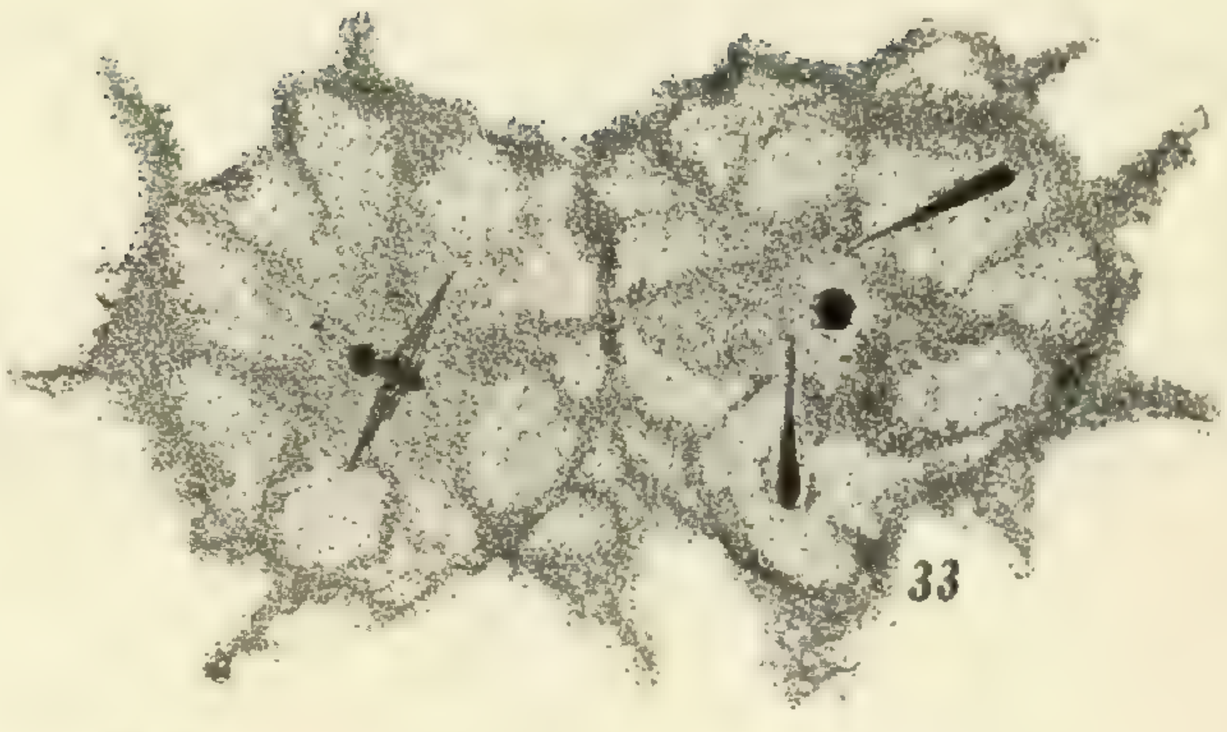
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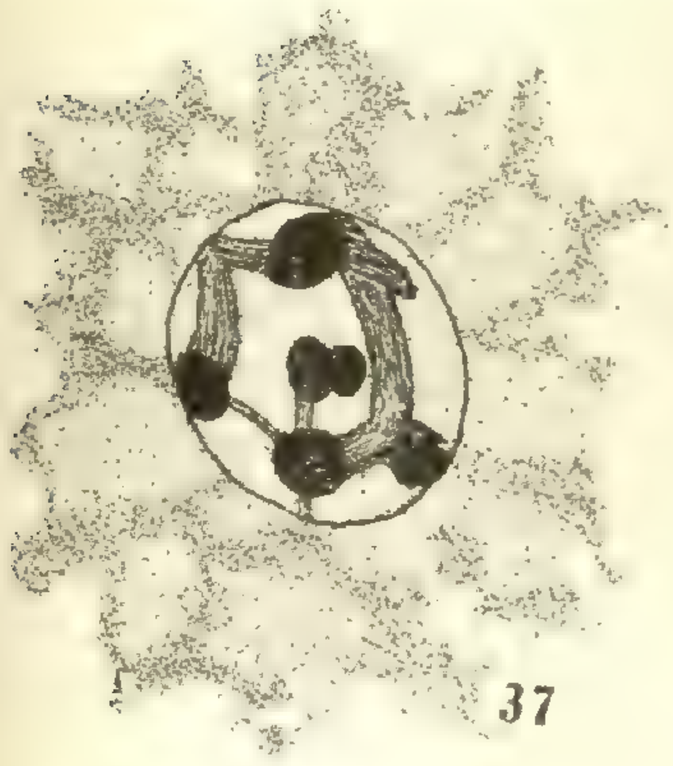
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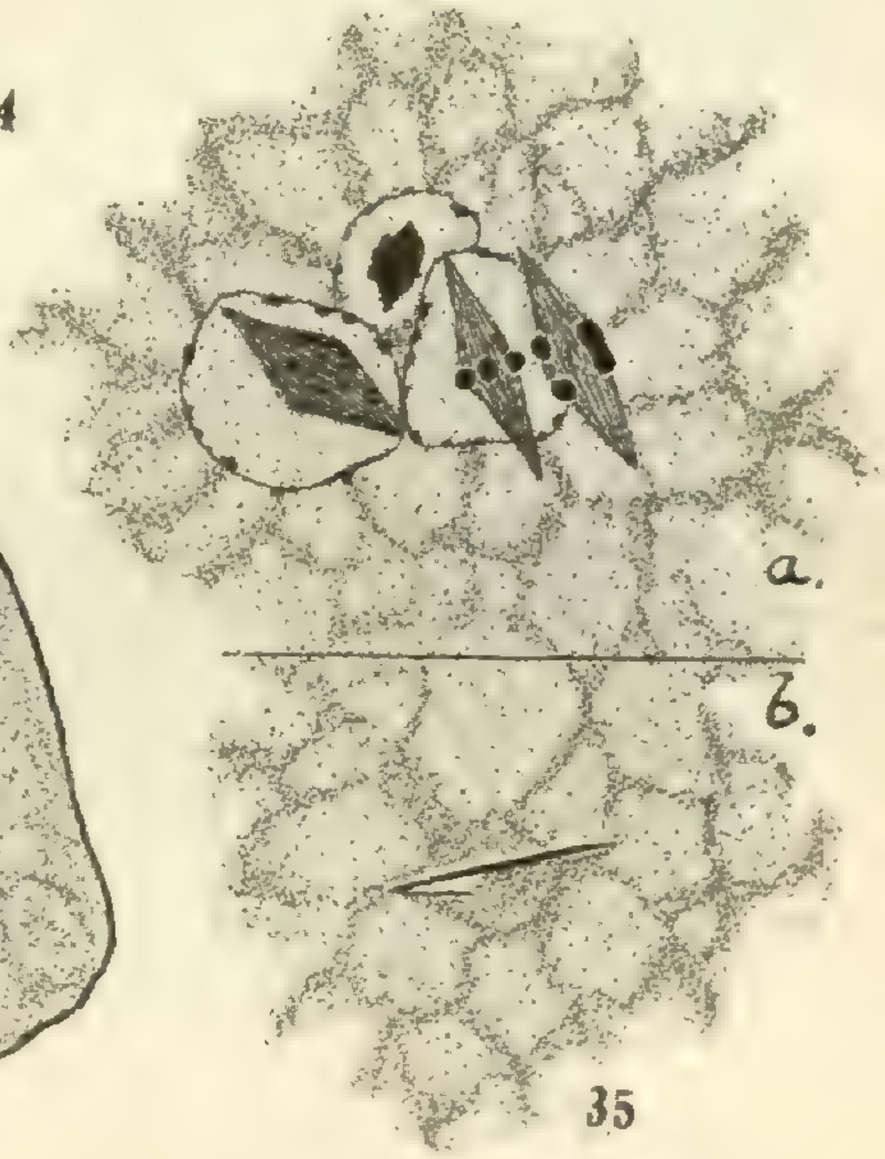

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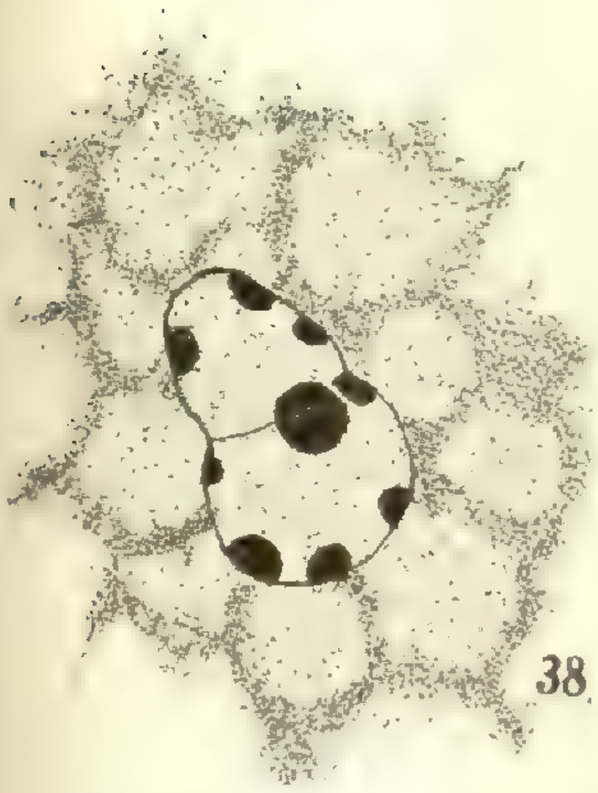
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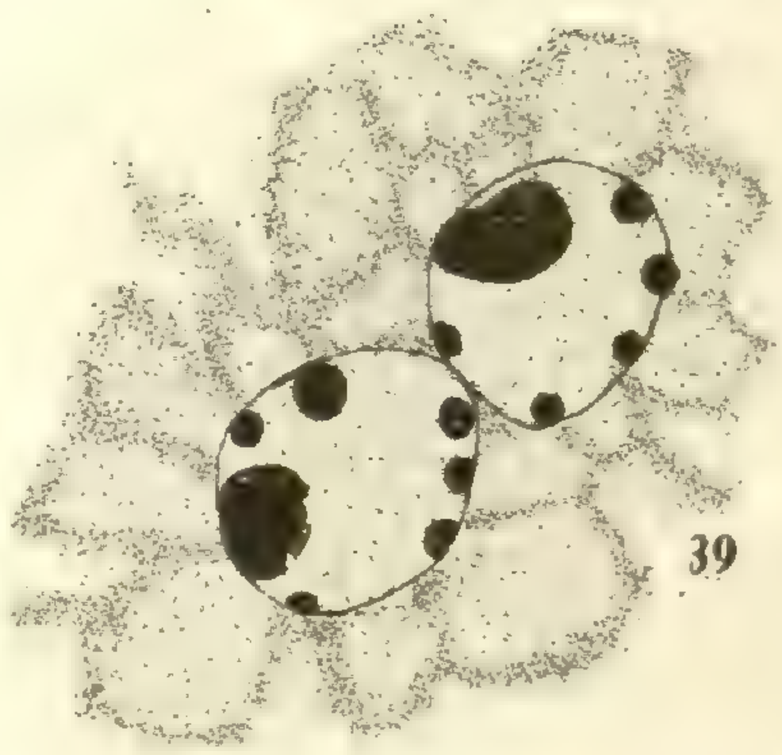
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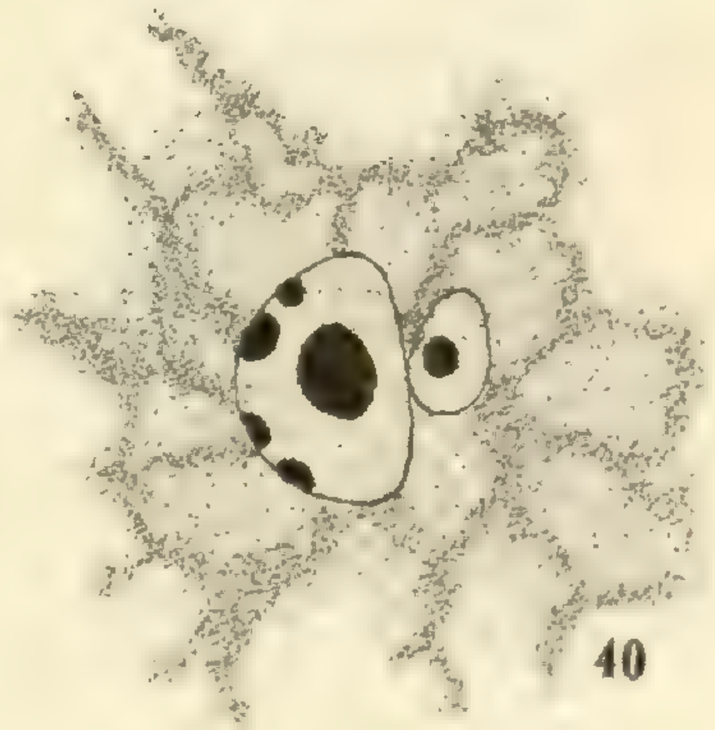
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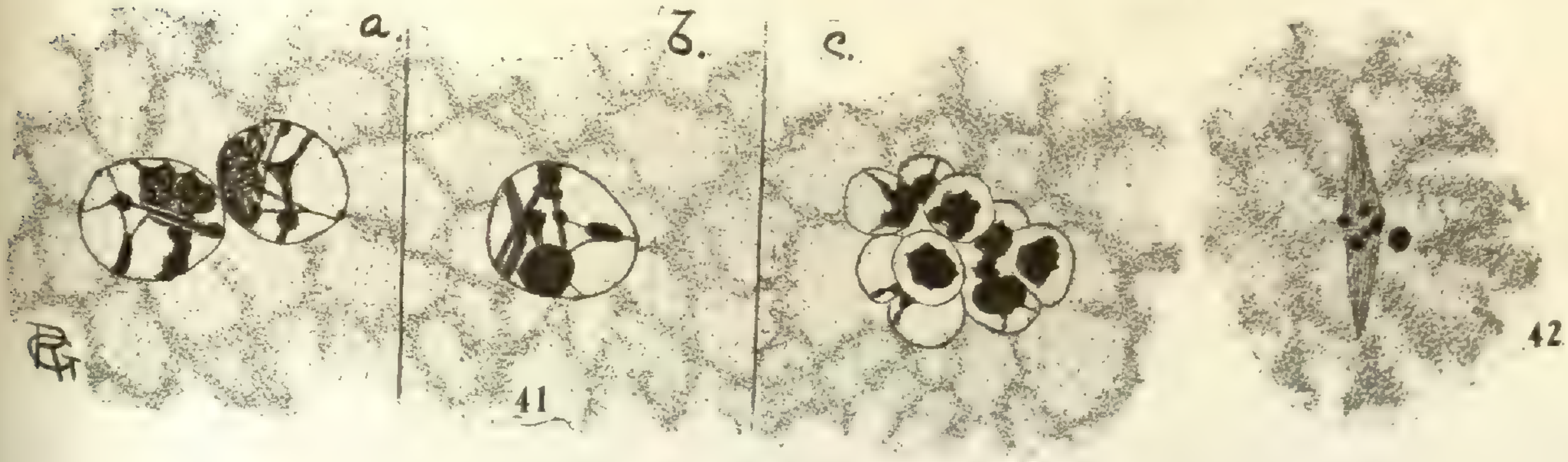

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INFLUENCE OF ELECTRICITY ON MICRO-ORGANISMS

GEORGE E. STONE

(WITH TWO FIGURES)

The influence of electricity on the higher plants has been studied for many years, and there is considerable literature pertaining to this subject. The writer has carried on investigations in this line for many years, and some of the results have been published from time to time.

Little or no attention, so far as we know, has been given to the study of the influence of electricity on the growth and multiplication of microorganisms, and it is our purpose to present in this paper the results of some of our investigations of the past two or three years.

Microorganisms are favorable types with which to experiment, since they respond very quickly to stimuli, and, as might be expected, the results are more pronounced than is the case with the higher plants, where growth is relatively slower. The investigations given in this paper were made on microorganisms common to water, milk, and soils, and some experiments were made with yeast. In some instances the natural flora common to water, milk, soils, etc., was used, and in others we experimented with pure cultures.

Influence of electricity on bacteria in water

Our first experiments with the influence of electricity on microorganisms were undertaken in connection with those common to water, and were designed with the object of rendering stagnant water more wholesome by a system of electrical treatment. Our studies had not extended very far, however, before we found that, instead of being decreased by means of this treatment, the bacteria increased enormously, especially when weak currents were employed. In this series we made use of the natural bacterial flora of water, while in others isolated species were experimented with. The experiments were made in glass jars, in some cases those of rectangular form being used, and in others a wide-mouthed bottle. For the purpose of measuring currents we made use of a Weston milliammeter and the

usual bacterial methods were employed throughout these tests. All platings were made in Petri dishes in standard agar-agar, and the usual dilution methods were followed. The agar cultures were incubated at the usual temperatures, but the experiments were conducted at room temperatures in most cases, which ranged from 60° to 70° F.[1]

TABLE I

Showing the influence of electrical stimulation (galvanic currents) on the bacteria in water. First cultures made 24 hours after treatment.

DATE OF MAKING CULTURE	NUMBER OF BACTERIA IN 1^{cc}	
	Normal	Treated
June 19.....	3463	43,642
" 20.....	3435	108,785

The experiment shown in the preceding table was made with two rectangular jars with approximately the following interior dimensions: height 16^{cm}, width 12^{cm}, diameter 4^{cm}. These jars were filled with water obtained from a pond contaminated to a greater or less extent with sewerage. One was a normal or untreated jar, and the other contained electrodes composed of copper and zinc respectively, which were connected with wires and generated a current. The electrodes were of the same diameter as the jar, and one was placed in each end. A jar of this type constitutes a galvanic element (water cell), although the strength of current produced in this case is very weak, averaging about 0.1 milliamperes. Samples of the water were plated in agar-agar 24 hours after treatment. On the second day, however, the experiment was discontinued. The results given in table I show

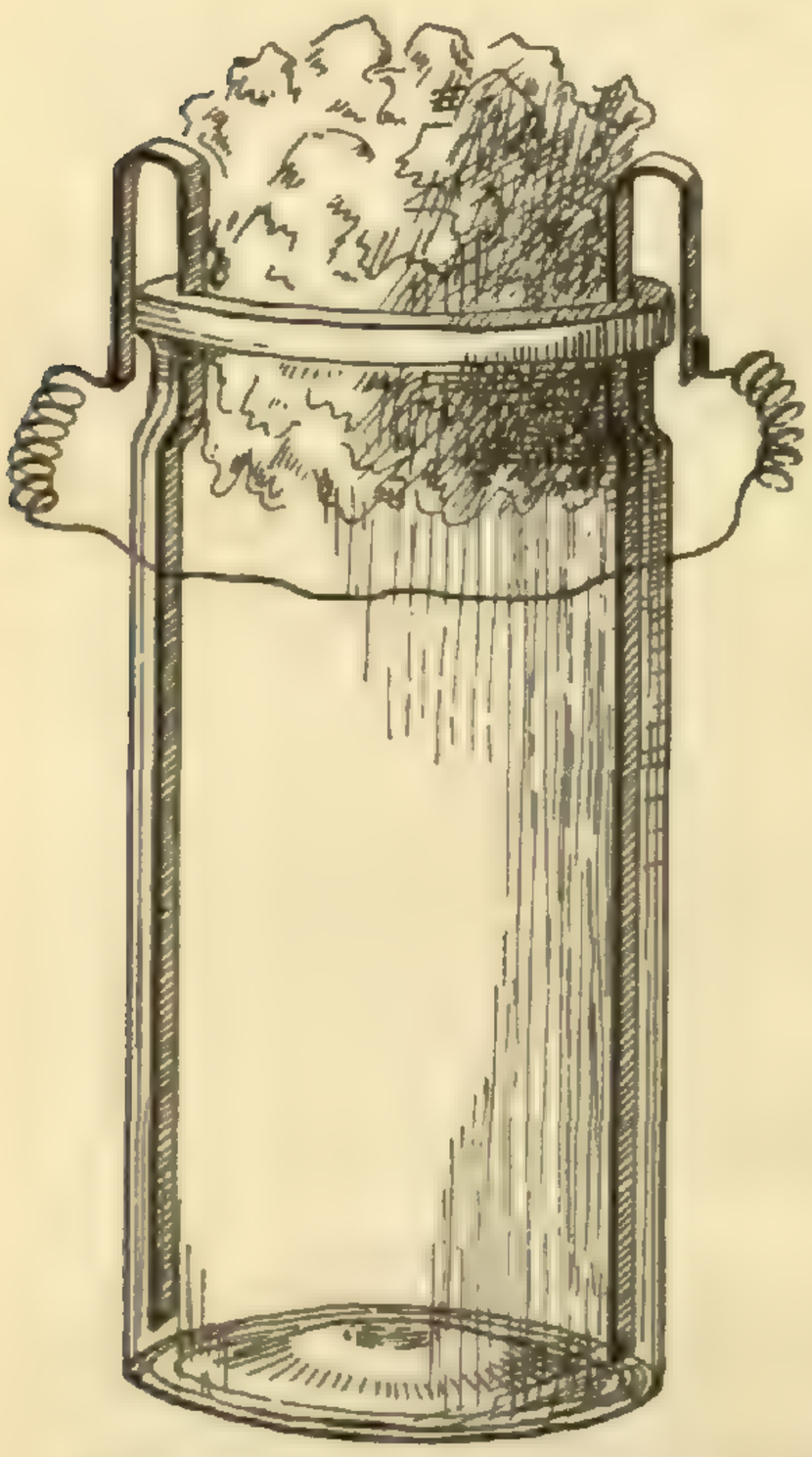

FIG. 1.—Jar, provided with cotton plug and copper and zinc electrodes, used in electrical experiments with milk and water.

[1] In carrying on these experiments the writer is under special obligation to Mr. N. F. MONAHAN, a former assistant in our laboratory, who supervised most of the details of the work.

considerable increase in the number of bacteria in water resulting from electrical stimulation.

The number of organisms in the electrically treated jar increased from about 3000 to 43,000 on the first day, and to 108,000 on the second day.

TABLE II

Showing the influence of electrical stimulation (galvanic currents) on *Pseudomonas radicicola*. First cultures made 24 hours after treatment.

Date of making culture	Number of bacteria in 1^{cc}	
	Normal	Electrical
January 23	6,000	15,000
January 24	50,893	3,178,246
January 27	52,741	4,287,002
January 31	50,217	5,210,112
February 4 ...	50,217	425,000
February 8 ...	42,112	10,200
February 12 ...	41,110	50,000
February 16 ...	35,000	4,000

TABLE III

Showing the influence of electrical stimulation (galvanic currents) on *Bacillus megaterium* DeBary. First cultures made 24 hours after treatment.

Date of making culture	Number of bacteria in 1^{cc}	
	Normal	Electrical
February 25 ...	11,000	243,000
February 28 ...	21,000	3,462,000
March 4.....	25,400	5,600,000
March 8.....	20,000	4,566,400
March 12.....	32,000	7,650,000
March 16.....	10,000	243,000
March 20.....	35,000	500,000
March 24.....	22,000	22,000

The experiments given in the preceding tables were made in wide-mouthed jars approximately 10^{cm} in diameter and 21^{cm} high (*fig. 1*). Those containing the electrically treated water were provided with electrodes made of copper and zinc, which were connected with a wire as in the last experiment. The electrodes were about 4^{cm} wide and long enough to extend over the lip of the bottle. The strength of current developed in this galvanic cell was about 0.3 milliamperes, and it remained very constant throughout the experiments. The

jars in every case were provided with cotton plugs and the whole outfit was sterilized before using. In these experiments pure cultures were used, and the medium, in this case water, was also sterilized before being inoculated. In one series (table II) the jar was inoculated with *Pseudomonas radicicola* (Beyerinck) Moore, from alfalfa; while in the experiments shown in table III the jar was inoculated with *Bacillus megaterium* DeBary, 1^{cc} of a liquid culture medium being used to inoculate the jars in each case. Special care was taken to inoculate the normal and treated jars with the same number of organisms. An examination of the tables II and III will show that there was a marked increase in the number of bacteria during the first few days as a result of electrical stimulation. The maximum in one case was 52,000 for the normal and 5,000,000 for the treated; in another case 32,000 for the normal and 7,000,000 for the treated. It will be noted that the subsequent decrease in the number of the organisms was very marked in the electrically stimulated cultures, a feature due to the accumulation of zinc oxid in the jar, which is always present as a white precipitate in galvanic cells of this type. The presence of zinc oxid in water formed by the action of even comparatively weak currents is toxic to bacteria, and the same toxic effect is well illustrated in galvanotropic experiments with roots. Some of our experiments which were made in much smaller jars failed entirely, as the smaller volume of water employed became concentrated so quickly with this substance that a toxic effect on the organism occurred very shortly after inoculation. Some of the precipitate obtained was dried and dissolved in flasks containing sterilized water. The jars were then inoculated with *Bacillus megaterium* DeBary, with the result that very little increase in the number of bacteria occurred where a 2 per cent. solution of this prepared precipitate of zinc oxid was used, and a 10 per cent. solution apparently killed all bacteria. In both of the experiments enumerated there occurred a slight falling-off in the number of organisms in the normal or untreated cultures. This is of common occurrence, however, in standing water, or even in soils under certain conditions.

The strength of current developed in these experiments (0.1 and 0.3 milliampere) was very constant, and from the results obtained it is evident that it acted as a marked stimulus. A large series of

experiments made by us on the higher plants has shown that this current strength is very close to the optimum, and in all probability the optimum current strength for bacteria would differ little if any from that of the higher plants. We have employed this method of electrically stimulating bacteria and have enormously increased the number of organisms in cultures containing the legume Pseudomonas, which is used in inoculating soils.

Some experiments were also carried on at the same time relative to the influence which electrical stimulation might have upon nitrogen fixation, but the results are incomplete and will not be given in this paper.

The influence of electricity on bacteria in milk

The purpose of our experiments in this series was similar to that in the experiments made with water; that is, to determine the effect of electrical stimulation on the microorganisms in milk. Our object, however, was not only to ascertain the effects of optimum currents, or at least those approximating the optimum on the bacteria of milk, but to observe the effects of strong electrical charges.

Milk constitutes an excellent medium for the multiplication of bacteria and is well suited in some respects to experiments of this nature. The experiments given in tables IV and V were conducted similarly to the ones shown in the preceding series; that is, the bacteria were stimulated by galvanic currents and the same size culture jars were used (*fig. 1*). About 1.5 pints of unsterilized milk were placed in each jar and a milliammeter indicated the strength of current to be approximately 0.3 milliampere in the electrically treated samples. The jars were provided with cotton plugs and were sterilized before being filled with milk. The usual dilution methods were followed and the standard agar-agar was used for plate cultures. In practically all instances the counts are averages of three and four plates. Platings were made of the milk at the beginning of the experiment, that is, before being electrically stimulated; therefore these counts, which are averages, answer for both the treated and untreated cultures.

The results of electrical stimulation on bacteria in milk are shown in the experiments given in tables IV and V, but since milk sours and curdles badly in a few days it was necessary to limit the duration

of the experiments. In the normal or untreated samples the increase in the number of organisms in one experiment was from 143,000 to 6,000,000; while in the treated samples the number reached 94,000,000. In the experiment shown in table V the normal increased from 118,000 to 4,000,000; while the electrically treated reached 83,000,000. The results are more striking than those obtained by the treatment of water, as might be expected, since there was more food available for the use of the organisms in the latter series.

TABLE IV

Showing the influence of electricity (galvanic currents) on the bacteria in milk

Date of making culture	Number of bacteria in 1cc	
	Normal	Electrical
May 16, 2 P. M.....	143,395	143,395
May 17, 9 A. M.....	809,112	3,874,421
May 17, 5 P. M.....	1,470,441	86,592,600
May 18, 9 A. M.....	6,082,542*	94,851,806*

* Milk sour.

TABLE V

Showing the influence of electricity (galvanic currents) on the bacteria of milk

Date of making culture	Number of bacteria in 1cc	
	Normal	Electrical
May 17, 10 A. M.....	118,542	118,542
May 17, 5 P. M.....	678,333	1,848,806
May 18, 10 A. M.....	1,026,533	41,778,766
May 18, 5 P. M.....	4,591,500*	83,363,866*

* Milk sour.

Another series of experiments was undertaken to demonstrate the effects of static electricity on bacteria in milk. For this purpose we employed a static machine of the Töpler-Holtz type, which was designed for X-ray work and is capable of producing a spark six inches or more in length. The method of plating, etc., was the same as has been previously described, and the culture jars were of the same type, except that the electrically stimulated jars were covered with tinfoil in the same way as a Leyden jar. The copper and zinc plates were dispensed with, of course, and the milk was charged direct from the static machine. In this series three jars were used:

one normal, one treated with positive, and one with negative charges. The milk in the electrically treated jars was charged with sparks from a static machine in the same way that a Leyden jar is charged, an electroscope being used to determine the nature of the charge. In the milk experiments which follow it should be pointed out that the charges varied considerably.

TABLE VI

Showing the influence of static electricity (positive and negative charges) upon the bacteria in milk. Electrical jars charged with one spark each June 11.

Date of making culture	Number of bacteria in 1cc		
	Normal	Electrical (positive charge)	Electrical (negative charge)
June 11	8,342	8,342	8,342
June 12	69,000	196,300	210,000
June 13	568,000	1,240,000	1,367,500
June 14	1,213,000	16,432,500	19,374,600 ✓
June 15	9,876,400	70,500,000	79,600,000
June 16	27,432,000	153,461,000	131,540,000
June 17	190,500,000*	267,000,000*	233,330,000*

*Milk curdled.

TABLE VII

Showing the influence of static electricity (positive and negative charges) upon the bacteria in milk. Electrical jars charged with 10 sparks each June 2.

Date of making culture	Number of bacteria in 1cc		
	Normal	Electrical (positive charge)	Electrical (negative charge)
June 2, 10 A. M	565,000	565,000	565,000
June 2, 5 P. M	1,173,000	597,666	624,333
June 3, 10 A. M	19,057,000	23,443,666	18,088,333
June 3, 5 P. M	107,440,000	151,516,000	125,592,000
June 4, 10 A. M	201,413,333*	287,380,000*	212,816,666*

*Milk sour.

In the experiment shown in table VI one large spark was given each electrically treated jar, one being given a positive and the other a negative charge, and in the one shown in table VII the number of sparks was increased to 10. The electrical treatment, the results of which are shown in table VI, where the milk was charged with a single spark and cultures made every 24 hours, gave rise to a decided acceleration. This acceleration was perceptible at the time of the

first plating and continued throughout the experiments, which lasted six days.

The results shown in table VII indicate that the treatment given caused less acceleration. In this case platings were made at shorter intervals. The first plating was made seven hours after stimulating; at this time scarcely any acceleration was shown, which indicated the possibility of the death of the organisms by treatment, while during the same period the normal nearly doubled in the number of bacteria. The later platings, however, made at 24, 31, and 48 hours respectively after stimulation, showed a greater increase than that given by the normal, but the ten charges given in the latter experiment were evidently too strong to obtain the optimum results. In both the experiments enumerated (tables VI and VII) the milk was kept on ice.

TABLE VIII

Showing the influence of static electricity (positive and negative charges) upon the bacteria in milk. Electrical jars charged with 100 sparks each.

DATE OF MAKING CULTURE	NUMBER OF BACTERIA IN 1CC		
	Normal	Electrical (positive charge)	Electrical (negative charge)
May 31, 10 A. M.....	528,000	528,000	528,000
May 31, 5 P. M.....	1,546,000	568,333	601,100
June 1, 10 A. M.....	24,885,344	1,323,333	916,666
June 1, 5 P. M.....	164,033,000	2,144,600	2,133,460
June 2, 10 A. M.....	225,103,632	15,068,333	13,631,090
June 2, 5 P. M.....	200,500,000	84,654,000	45,612,000
June 3, 10 A. M.....	149,930,000	102,032,420	83,533,220

In another experiment (table VIII) the number of sparks was increased to 100 and the electrically treated jars were charged at 10 A. M. and 5 P. M. each day for a period of three days, with the result that a decided inhibitory effect was noticed after the first treatment, followed by a less increase for the succeeding periods than that given by the normal, and on the third day, when the experiment was discontinued, the electrically treated milk showed a smaller number of bacteria than the normal, thus showing that electrical charges were too strong for the maximum development of the organisms.

A much greater inhibitory effect is shown in table IX, where 100 sparks were applied at more frequent intervals—10 A. M., 1 P. M.,

and 5 P. M. respectively for two days. At the close of the experiment there was little or no difference between the normal and the treated samples. The subsequent charges, however, failed to prevent an increase in the number of bacteria.

TABLE IX

Showing the influence of electricity (positive and negative charges) on the bacteria in milk. Electrical jars charged with 100 sparks each 10 A. M. and 1 P. M. and 5 P. M. each day.

Date of making culture	Number of bacteria in 1cc		
	Normal	Electrical (positive charge)	Electrical (negative charge)
July 3, 10 A. M.....	5,580	5,580	5,580
July 3, 1 P. M.....	21,440	381	404
July 3, 5 P. M.....	78,533	380	487
July 4, 10 A. M.....	268,500	5,664	10,367
July 4, 1 P. M.....	863,830	10,806	26,990
July 5, 10 A. M.....	17,800,000*	19,180,000*	19,910,000*

* Milk sour.

TABLE X

Showing the influence of electricity (positive and negative charges) on the bacteria in milk. Electrical jars charged with 50–100 sparks each every hour (10 A. M. to 5 P. M. inclusive).

Date of making culture	Number of bacteria in 1cc		
	Normal	Positive	Negative
July 6, 10 A. M....	219,250	219,250	219,250
July 6, 3 P. M....	1,000,000	481	266
July 6, 5:30 P. M....	10,655,000*	11,522*	935*

* Sour.

As it was one of the objects of these experiments with milk to determine the maximum stimulus, the charges were increased. For this reason the experiment shown in table X was made, where an even more marked falling-off in the number of organisms is shown. The experiment lasted only a few hours, and was not continued on account of the souring of the milk.

The hourly charges of 50 to 100 large sparks, however, did not entirely prevent the organisms from developing, but the falling-off from over 200,000 to a few hundred in 1 cc was significant.

In another experiment, where only one series of heavy charges was given, the number of organisms decreased from 31,000 to 35 one-half hour after treatment; while on the third day the number in the normal was 29,000,000 against 1,000,000 of the treated. Further experiments of a similar nature were made, but in no case were we able to prevent the subsequent appearance of organisms.

The immediate falling-off in the number of bacteria when strong and frequent charges from a static machine were employed would point to the conclusion that the electrical treatment destroyed the bacteria, but what effect it may have had on the spores was not determined. The ultimate increase in the number of organisms in every case after treatment, where heavy charges were used, is also significant, and may be explained on the supposition that strong electrical charges did not affect the spores; in other words, the immediate falling-off in numbers resulting from excessive stimulation may be due to the destruction of the vegetative forms, and those which did appear in the agar cultures may have developed entirely from spores which were possibly not affected detrimentally by treatment. Furthermore, there is a possibility that the strong static charges might induce a tendency in the organisms to spore formation, and the spores, not being affected by the heavy charging, would germinate in the agar cultures.

In the experiment shown in table IX, where a considerable falling-off in the number of organisms took place at first, which was followed by subsequent increase, there was a long period in the night when no stimulation was applied, and spore formation may have taken place; but in the experiment shown in table X the period elapsing between treatments was only one hour, yet the number of bacteria in one instance increased very perceptibly; namely, in the positively charged culture. On the other hand, if the case is one of inhibition or suppression of the vital processes only, we should expect this effect to be lost in the period elapsing between plating and the counting of the colonies. The possibility of accommodation or adaptation of the organisms to intense stimulation is also not out of the question, as this frequently occurs. The roots of certain plants, for example, can grow and develop in water saturated with poisonous gases if given an opportunity to adapt themselves to these extreme conditions,

whereas these plants, if developed normally and placed under these conditions, would suddenly collapse. Our experiments do not furnish sufficient data to determine which of these views is correct. The maximum stimulation would undoubtedly be different for spores than for bacteria existing in the vegetative stage, as it is well known that those in the vegetative stage succumb more readily to heat than those in the spore form. The effects of electricity on spores can best be determined by experiments with pure cultures possessing certain characteristics, rather than those of heterogeneous types such as characterized the flora of the milk with which we experimented.

In our experiments with milk and water, where galvanic currents were employed, the stimulus was constant, whereas when only one shock or a series of shocks was given the organism from a static machine the charges soon disappeared, although the effects of electrical stimuli of brief duration give rise to decided reactions.

It should be pointed out that an increase of even twenty fold in the number of organisms in a given solution at the outset would make a vast difference in the number a few days later, even if the same subsequent rate of increase followed in both the normal and treated cultures. The amount of available food supply in a solution, however, is limited, and in the end there is often little difference in the number of organisms present in any treated or untreated series.

Undoubtedly the use of strong electrical currents is capable of destroying bacteria and preventing milk from deteriorating, although other methods of electrical treatment would probably prove more satisfactory than those which we employed. In some tests made of electrically treated milk we found that souring was delayed. It is well to note in passing that there appears to be a difference in the effects of the positive and negative charges; for example, if a comparison is made of the averages of the last counts in tables VI to X inclusive, it will be found that the milk treated with positive charges gave a larger count than that treated with negative charges. The number of bacteria shown in the positively charged jars was 135,000,000, while that of the negatively charged was 109,918,164. This is what might be expected, since the writer has previously demonstrated in a large series of experiments on the growth of seedlings that positive charges stimulated both roots and stems more than the negative charges.

Feeble electrical currents and small static charges act as stimuli to bacteria in milk, increasing their numbers very perceptibly, and under certain conditions there may be after all some foundation for the old belief that milk sours more quickly during thunderstorms than at other times. Notwithstanding the fact that there may be other conditions during thunderstorms, such as warm and sultry weather, which may accelerate bacterial action, it is not difficult to imagine conditions under which milk might be stored which would subject it to electrical stimulation, thus increasing the number of bacteria and incidentally hastening souring.

The influence of electricity on bacteria in soils

Only a limited number of experiments was made by us relative to the influence of electricity on bacteria in soils. Careful bacterial analyses of soils are tedious operations and the methods followed in these analyses were similar to those recommended and used by CHESTER.[2] In all cases the counts were made on one gram of air-dried soil. In these experiments we made use of wooden boxes 8×8×8 inches, inside measurements, which were filled with fairly good loam free from coarse material. The soil used was more or less compact, very fine sand (0.01^{mm}—0.005) predominating. It might be expected that the texture of the soil, as determined by the size of the particles, the amount of organic matter, and plant food, would exert an important influence on the bacterial flora, and the rather fine texture of the soil which we selected for this experiment would give results different from those that would be obtained from a looser soil containing larger particles and having more air space, or one containing a larger amount of organic matter.

In the box electrically treated were placed copper and zinc electrodes, each being 8×8 inches in size, and to these were soldered copper wires which were connected, thus forming with the soil a galvanic cell which furnishes a small current approximating the optimum. The percentage of water in the soil in each box was accurately determined at the beginning of the experiment, and this same percentage was maintained throughout by adding sterilized distilled water. The experiments were carried on in the laboratory under conditions as

[2] CHESTER, F. D., Delaware Agric. Exper. Sta. Bull. 65 : 61–65. 1904.

nearly identical as it was possible to make them, the soil being exposed to the air continually. The cultures were plated in agar-agar and the usual dilution methods were followed.

TABLE XI

Showing the results of electrical stimulation (galvanic currents) on the bacteria in soil.

	Date of sampling	Number of bacteria in 1gm	
		Normal	Electrical
Experiment I.....	July 13	33,470,000	37,930,000
	July 29	28,777,000	32,863,000
	August 11	19,294,000	35,000,000
Experiment II.....	September 14	38,047,000	37,670,000
	October 16	18,720,000	26,384,000

Two experiments are given in this table, the first date of sampling corresponding with the beginning of the experiment. No attempt was made to disturb or cultivate the soil in the boxes, and the surface became more or less compacted by constant watering, which no doubt accounts for the general falling-off in the number of bacteria in all cases. It will be noted that no increase is shown in the number of bacteria in either the treated or untreated soils, although the extent of falling-off in both experiments is less in the electrically treated boxes. Similar experiments were made with soil in the same boxes with static electricity. The boxes in this case were arranged as follows: one box was left normal as before, and the other had 12 wires extending into the soil leading to a metal bulb which was given 100 sparks from a Töpler-Holtz machine once a week. In this case the first samples were taken a few days after electrical treatment; otherwise similar methods were employed as in the preceding series.

In this case there did not occur the same falling-off or decrease in the number of the organisms in either the treated or untreated cultures at the time of the subsequent platings, as in the preceding series. On the other hand, the treated soil in this experiment showed a continual increase in the number of bacteria present at the time of the different platings. This was apparently due to the frequent stirring or cultivation of the soil. The number of organisms, however, showed only a slight increase at the time of the first platings, whereas the

electrically treated increased from 4,000,000 to 27,000,000. The soil in this case was freshly prepared and carefully mixed and contained more organic matter and plant food than the former; neither was there the same tendency for the soil to become badly compacted as in the preceding series.

TABLE XII

Showing the results of electrical stimulation on the bacteria in soil (static electricity).

Date of sampling	Number of bacteria in 1^{gm}	
	Normal	Electrical
July 21.....	1,097,290	4,506,700
July 31.....	960,000	15,208,000
August 7.....	1,960,780	27,756,000

The electrical experiments with soil were not continued, since the details associated with soil bacteriological analyses are laborious. The results of electrical stimulation of soil organisms are not so pronounced as in the case of the water and milk experiments, but the effects are clearly shown in table XII.

Our numerous experiments in growing plants in electrically stimulated soils have demonstrated that considerable acceleration in germination and growth follow when currents of optimum intensity (0.1—0.6 milliampere) are employed, and all the forms of plant life are undoubtedly stimulated in a similar way.

Influence of electrical stimulation on yeast

Some experiments were made in our laboratory for the purpose of observing the effects of electrical stimulation on yeast, in which we endeavored to determine the relative activity of the normal and electrically treated organisms by the amount of CO_2 given off.

As in the preceding series, we used galvanic currents obtained by the use of copper and zinc electrodes and also static charges from a Töpler-Holtz machine. Small bottles were used in the experiments, ranging from 2 to 4^{cm} in diameter and 12 to 16^{cm} high. The electrodes were 2^{cm} in diameter and were placed in the bottle containing the yeast and culture media. In the experiment with static electricity we made use of simple Leyden jars corresponding to those described

in the preceding series. In all instances corresponding jars were employed in the normal and electrically treated yeast, and the conditions were identical so far as they could be made in every way in all cases. From 0.5 to 5^{gm} or more of yeast were placed in each jar in the various experiments, and either a solution of molasses (about 5 per cent.) or a standard nutrient solution was used. The nutrient solution was made up as follows:

	Grams
Calcium nitrate	6.0
Potassium nitrate	1.5
Magnesium sulfate	1.5
Neutral potassium phosphate	1.5
Sodium chlorid	1.5
Cane sugar	125.0
Distilled water	2500.0

This solution was not chosen on account of its being the best adapted for this purpose, but it proved to be satisfactory.

The yeast used in these experiments was from the ordinary commercial yeast cakes, which were cut into cubes and carefully weighed. The yeast was then put into mortars containing the nutrient solution and the cells carefully separated by repeated stirring. After the yeast cells were well separated they were placed in their respective bottles. The mortar was thoroughly rinsed and care was taken to have the same amount of yeast in each. The bottles containing the yeast were completely filled and connected by means of glass tubes to graduated cylinders or burettes containing water, and as CO_2 was given off, the displacing of water was noted at intervals and recorded. The following table gives the results of some experiments with yeast.

The experiments (see table XIII) had a duration of 1.5 hours to 4 days, and in all instances the amount of CO_2 given off was greater in the electrically treated than in the normal or those not stimulated. The experiments were conducted on different days and at different temperatures, most of them being at room temperatures, and as there was no heat on in the laboratory at the season of the year in which many of these experiments were conducted, the temperature was naturally somewhat variable. In *a*, *b*, *c*, *g*, and *h*, however, the bottles containing the yeast were in water baths and were kept under conditions nearer to the optimum for yeast (32—38° C.), hence the per-

centage of CO_2 given off in these was greater for a given period than in some of the others. All of the other experiments were run at room temperatures, which in some instances were fairly good, and in others the room was too cool to expect much activity on the part of the yeast. The small amount of CO_2 given off in some instances is therefore due to the temperature conditions under which the experiment was made, and moreover, since these experiments in some instances lasted four days, there was more or less absorption of CO_2 by water in the graduated cylinders.

TABLE XIII

Showing the influence of electricity on yeast

Experiment	Nature of electrical stimulus	Duration of experiment	Number of cc of CO_2 given off in	
			Normal	Electrical
a	galvanic	2 hours	174	212
b	galvanic	3 days	752	838
c	galvanic	2 hours	189	232
d	galvanic	3 days	540	700
e	galvanic	4 days	20	296
f	galvanic	4 days	50	1200
g	static*	1.5 hours	112	129
h	static	2 hours	74	102
i	static	7 hours	82	120
j	static	3 days	922	1035
k	static	2 days	300	575
l	static	4 days	15	140

* *g* was given 8 sparks; *j* and *l*, 5 each; *k*, 1 spark; *h*, 2; and *i*, 3.

The absorption of CO_2 by water was noticeable when the yeast cultures were left over night, especially when the room temperature was low and the yeast not active. The absorption of water, however, was apparently the same in both the treated and untreated series and did not affect the relative results. In some cases, therefore, a larger amount of CO_2 was given off than is shown by the records in the tables.

Frequent observations and readings were made of the amount of CO_2 given off, but to make the tables brief we have given only the final readings.

The effects of electrical stimulation seem to be more pronounced in the lower temperature experiments than in those where there was a high temperature. In some instances the untreated cultures gave

off very little gas, while the treated, under the same temperature conditions, gave off considerable. The electrical stimulation, under these conditions, appeared to act very much as yeast would if subject to an increase in temperature.

In those experiments where the carbon dioxid gas readings were made at short and regular intervals, we were able to observe the effects of the stimulation on the organisms at different periods.

In some experiments the observations were made every five minutes from the start, and after a number of observations were made and recorded, the stimulus was applied. The results obtained in one of the experiments, in which five-minute observations were made, are given in the curve shown in *fig. 2*.[3] This curve is based on the increased amount of carbon dioxid given off by the normal or untreated; in other words, the amount of CO_2 generated by the normal in this case would be represented by the base line, or it would be equivalent to zero. The treated yeast was given two very small sparks from a static machine at 1:50 P. M., or one-half hour after the experiment was started. A temperature of 30—35° C was maintained during the experiment.

The relative amount of CO_2 given off by the electrically treated and untreated yeast before the stimulation was applied showed quite a uniform activity on the part of the yeast. Following the latent period, which is usually of 15 to 25 minutes' duration, the results of the treatment were manifested in considerable acceleration in gas production. This reached its maximum effect at 3:20 P. M., or 1.5 hours after the stimulation had been applied. Another stimulus consisting of two minute sparks from a Töpler-Holtz machine was applied at 3:45 P. M. and produced a brief reaction, as shown by the curve. The experiment, however, was discontinued at 4:20 P. M.

In the experiments with yeast there was considerable variation in the number and size of the sparks applied to the treated jars, and there appears to be an indication, from the results obtained, that in some instances the electrical charge was too severe. This was noticed in the short-interval readings immediately following the stimulation, and in such cases the maximum acceleration period was more remote

[3] We are indebted to Mr. G. H. CHAPMAN, assistant in the laboratory, for supervising this experiment, as well as one other in this series.

from the stimulation period than in this case, where very weak charges were used. A charge of one or two minute sparks from a Leyden jar seemed to cause the most active response on the part of the yeast.

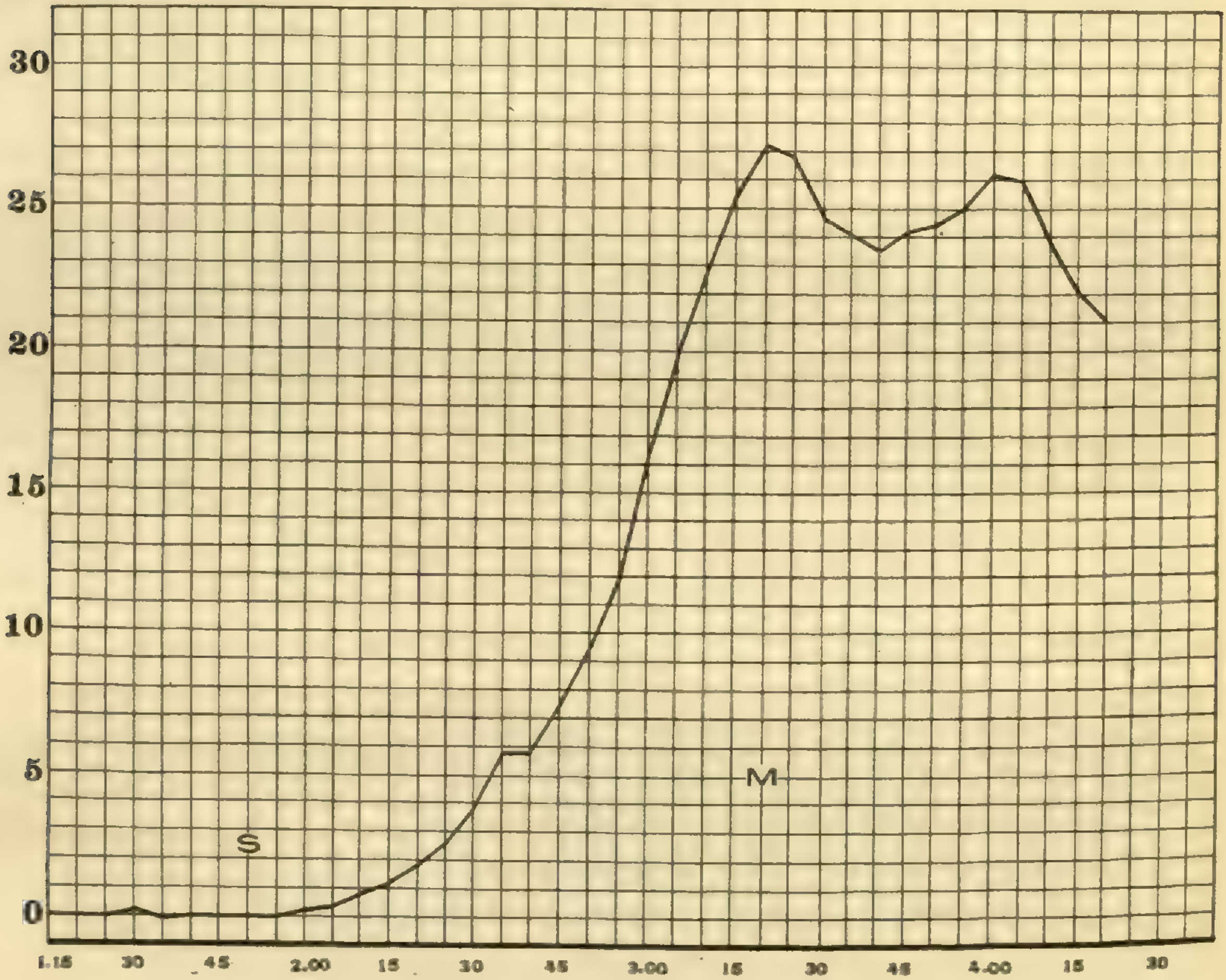

FIG. 2.—Curve representing the amount of CO_2 given off by electrically stimulated yeast; the horizontal lines represent the amount of carbon dioxid in cubic centimeters; the vertical lines five-minute periods of observation; *S*, time stimulation was applied (1:50 P. M.); *M*, maximum effect of stimulus.

Discussion of results

In considering the results presented here it should be pointed out that little attempt was made to ascertain the strength of the current necessary to produce the best results, and the static charges employed differed in number and intensity. We have already demonstrated in a large series of experiments[4] with the higher plants that the optimum current for germination of seeds and growth of seedlings is not far from 0.1 milliampere, and in our study of the effect of static

[4] STONE, G. E., The influence of current electricity on plant growth. Ann. Rep. Hatch Exper. Sta. Mass. Agric. Coll. **16**: 13–31.

charges on the germination of seeds and growth of seedlings we have observed that a very few minute sparks from a static machine caused the most marked stimulation.

In regard to the influence of atmospheric electrical potential on growth, MONAHAN[5] found that when the air in a glass case is charged to a potential of about fifty volts, better results were obtained than when a higher potential was used. We endeavored, therefore, in these experiments to make use of current strengths approximating the optimum, or that strength which gave the best results in our previous investigations with various organisms, except in those cases where strong static charges were given to milk for the purpose of ascertaining the degree of stimulation which would kill the organisms.

The results obtained from these researches suggest many lines of work which might be followed, but we are obliged to discontinue them for the present. Electricity undoubtedly, in one way or another, plays a very important rôle in plant life. Seed germination and growth of seedlings are greatly accelerated by feeble currents, but, unlike amides and enzymes, they are incapable of affecting the germinating capacity or of regenerating, as it were, the life in the seeds. The roots of the higher plants exist in a medium which is charged negatively, and the electrical potential of the air is often quite high within the limits of large trees. The electrical potential under the foliage of a tree is less than that at corresponding heights in the free atmosphere.[6] When, however, there is no foliage, the electrical potential under the branches of trees corresponds to that of the free air at equal heights, and there is reason to believe that the apices of leaves are merely so many points for the gathering and discharge of electricity. Minute currents of electricity exist in plants, and it is known that during certain periods trees discharge sparks from the apices of the leaves, and trees may tend to equalize differences in potential existing between the earth and air. Rain drops in falling become electrically charged, and as they gather microorganisms in their descent through the air, these also probably become affected. The remarkable influence of rain upon

[5] MONAHAN, N. F., The influence of atmospheric electrical potential on plants. Ann. Rep. Hatch Exper. Sta. Mass. Agric. Coll. **16**:31–37.

[6] STONE, G. E., AND MONAHAN, N. F., Ann. Rep. Hatch Exper. Sta. Mass. Agric. Coll. **17**:13–31.

vegetation cannot be satisfactorily explained, in our opinion, by chemical analysis or by the various other conditions which prevail, and the idea that electrical stimulation plays an important rôle here is not an improbable one. It is also not unlikely that during thunderstorms the bacteria in milk are affected, although two series of experiments made by us in exposing milk in sterilized metal vessels at different elevations, where the electrical potential showed considerable variation, were by no means conclusive.

The effects of electrical stimulation on plant growth resemble more nearly those produced by heat, that is, in the tendency of the plant to assume a rather spindling growth; but this similarity in the method of reacting does not necessarily prove that electricity and heat are identical, since spindling growths in plants occur from other causes. The effects of electrical stimulation do not resemble those induced by light, since light inhibits growth; on the other hand, they more closely resemble the effects induced by lack of light (partial etiolation) and other forms of stimulation which may be produced by various agencies. Electricity stimulates seeds very perceptibly, causing an acceleration in growth, and probably has the same effect on spores, and in this way the number of bacteria in solutions might be increased. The process of cell division of bacteria and the budding of yeast are undoubtedly stimulated by electricity, which would result in an increase in the number of organisms and an acceleration of the metabolic process.

The effects of electrical stimulation, like other types of stimuli, are manifested shortly after application. With a current of optimum intensity a latent period occurs when no effect is discernible, and this is followed by an acceleration in growth and development. The nature of the response is dependent upon the intensity of the stimulus as well as upon its duration; therefore to determine the period of duration of any particular response or its maximum period, the intensity and duration of the stimulus must be taken into consideration. Since the intensity and duration of the stimulus employed in these experiments differed materially, the response periods would also vary accordingly. As regards the manner in which electricity stimulates organisms, little can be said at the present time, and the problem is as difficult of solution as the manner in which light, etc., affect the organisms.

Many theories, however, in regard to the cause of the stimulating effect of electricity on plant growth have been advanced, some of which are hardly worthy of consideration, since they fail to meet the requirements of experiments, and we will not enter into a discussion of them here.

Electricity, like other forms of stimulation, such as light, heat, etc., undoubtedly affects the protoplasm of the plant, which causes certain metabolic processes to become active and accelerated growth results. In plants showing circulation and rotation of protoplasm, e. g., Chara, Nitella, etc., feeble electrical currents induce a more rapid streaming of the protoplasm, which is undoubtedly associated with greater metabolic activity, and it is not at all unlikely that changes of a similar nature take place in other organisms when subject to feeble electrical currents.

AMHERST, MASS.

CYTOLOGY OF CUTLERIA AND AGLAOZONIA

A PRELIMINARY PAPER

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 129

SHIGÉO YAMANOUCHI

This preliminary note gives a brief account of my cytological studies of *Cutleria multifida* Grev. and *Aglaozonia reptans* Kütz. The material was collected last winter and spring at Naples, where I occupied a table of the Carnegie Institution at the Zoological Station. The work was begun at Naples and was continued at the University of Chicago under the direction of Professor JOHN M. COULTER and Professor CHARLES J. CHAMBERLAIN, to whom I wish to acknowledge my great indebtedness for their suggestions and criticisms. Many points of cytological interest and importance, as well as the discussion of literature, will be presented in the full account to be published later.

Cutleria multifida

GAMETOGENESIS

Cutleria multifida is generally dioecious. The young thallus, 1–3^{mm} long and narrowly fan-shaped, presents no features to distinguish between the male and female plants. When the thallus has reached the stage for the formation of reproductive organs, the habit of the male plant is occasionally different from that of the female; but an extensive comparative study of the forms suggests that there is great variability in habit, so that it seems impossible to distinguish the two sexual individuals by any morphological character except that they bear as a rule exclusively either male or female organs.

VEGETATIVE MITOSIS IN BOTH MALE AND FEMALE INDIVIDUALS.—Both male and female plants, in good condition, always have a hairy growth at the tips of the multifid filaments of the thallus. Any part of the frond in vigorous growth is favorable for the study of vegetative mitosis, but details are more easily followed in the terminal hairs and in the superficial layer of the entire frond.

The cells in these regions are full of plastids, with usually a single nucleus in the center. The nucleus in the resting state is very small, generally about the size of the plastids or sometimes a little smaller. The network is so finely built that it is hard to recognize much chromatin in it. Neither centrosomes nor central bodies with or without radiations seem to be present.

In early prophase the nucleus increases in size until it is twice the diameter of the resting nucleus and occupies a greater part of the cell, pushing aside the numerous plastids toward the periphery. During the growth of the nucleus, there appear just inside of the membrane chromatin knots which are evidently worked out from the chromatin network by the rearrangement of the material. These chromatin knots, which are of course in continuation with less deeply stained chromatin fibrils, are variable in number at first, but gradually there appears a certain number of chromatin knots that are afterward detached from the chromatin fibrils and become chromosomes, 24 in number. The chromosomes after segmentation gradually assume a slightly elongated rod-form and become arranged at the equatorial plate.

A little before the equatorial plate stage, two kinoplasmic accumulations arise from the cytoplasm surrounding the nuclear membrane at two poles. A well-marked central body in the kinoplasmic mass occurs only at late metaphase. The chromosomes split longitudinally and half of each chromosome proceeds to each pole. During this entire process the spindle is intranuclear. At telophase the nuclear membrane disappears and the two sets of daughter chromosomes, in a state of close aggregation, are now surrounded by cytoplasm, and the formation of the nuclear membrane follows.

When the daughter nuclei are organized, the central spindle disappears completely. The cytoplasm lying between the two nuclei begins gradually to assume a coarse, irregular, alveolar structure, and the walls of the alveoli, probably after a change in their material, form a new cell plate.

Thus vegetative mitosis agrees in its essentials in both male and female plants.

FORMATION OF MALE GAMETES.—When the male plant is young, the surface of the thallus bears tufts of hairs here and there in some-

what regularly scattered spots. Later, with or without association of hairs, there are produced short filaments which afterward bear male gametangia. Both the filaments and the hairs arise from superficial cells of the thallus.

One of the superficial cells commences to grow more vigorously than the rest and a typical nuclear division takes place. Two or more subsequent divisions result in a short filament of three or more cells, the terminal one of which is destined to be a male gametangium initial, whose nucleus becomes considerably larger than is common in cases of vegetative mitosis. The mitoses which take place up to the formation of the male gametangium initial are typical and the number of chromosomes is 24.

The details of nuclear division are much more easily and distinctly followed in the gametangia. During the prophase of the first division in the gametangium initial, even before the segmentation of chromosomes, the nucleus is marked by two distinct kinoplasmic accumulations at the poles, and their position indicates the axis of the division. The formation of the cell plate between two daughter nuclei is sometimes much more delayed than in cases of vegetative mitosis.

Following the first division in the gametangium there are several cell divisions, the walls being somewhat perpendicular to one another; as a result there is formed the well-known male gametangium of Cutleria, composed of a great number (sometimes as many as 200) of mother cells, regularly arranged in vertical and horizontal tiers. During all of these successive divisions 24 chromosomes appear.

The nucleus, cytoplasm, and plastids in the mother cell undergo a certain peculiar change, and the whole contents of the mother cell enter into the formation of a male gamete. After the maturity of the gamete a tiny hole is developed in the peripheral wall of the mother cell, through which the gamete escapes.

FORMATION OF FEMALE GAMETES.—The formation of tufts of hairs and of filaments bearing female gametangia is similar to that already described for the male plant. The structure of the cells of the superficial layer is apparently like that of the male plant, and so far as the development of hairs and the behavior of nuclei are concerned, there seems to be no distinction; but the difference is remarkable when the superficial cell which is destined to form a female gametangium begins

to grow. The growth usually proceeds until the cell becomes two or more times as large as the corresponding cell of the male plant. Curiously enough the nuclear growth does not keep pace with that of the cell; in other words, the nucleus in the superficial cell at the time of division has almost the same dimensions as in the male plant, and this equality persists up to the formation of mother cells.

The first division of the superficial cell is followed by two or more divisions, which result in a short filament whose terminal cell becomes a female gametangium. A number of divisions in the gametangium produces eventually a structure composed of regularly arranged mother cells.

The nucleus and cytoplasm in the mother cell undergo changes similar to those of the male plant, and after a rearrangement of the plastids a female gamete is formed by the transformation of the whole protoplast. The female gamete, thus formed and containing 24 chromosomes, is discharged from the mother cell.

FERTILIZATION AND GERMINATION

As has been stated, the nuclei in both male and female gametes contain 24 chromosomes. When the female gamete loses its motility and becomes quiescent, a free swimming male gamete becomes attached to it and the union of the two protoplasts occurs. For the sake of brevity, the details of the fusion of the two nuclei, following the union of the gametes, will be omitted in this note.

The fusion nucleus in the common mass of male and female cytoplasm rests for a certain length of time. The first segmentation division takes place within twenty-four hours or less after the union of the gametes. So long as the fusion nucleus remains in the resting state, the round contour of the sporeling is still kept, but when the nucleus has begun to show the early prophase, there is noticed at once at a certain part of the sporeling a slight protuberance, and the cell wall of the protuberance is seen to be considerably thickened. The axis of the mitotic figure of the first segmentation is always parallel with the elongated direction.

The number of chromosomes appearing in the prophase is 48. When these chromosomes become arranged in the equatorial plate, the intranuclear figure is well marked between the kinoplasmic masses

at the poles. After the organization of daughter nuclei the central spindle disappears, and the formation of a cell plate at the expense of the cytoplasm begins only after the nuclei have grown to a considerable size. During the second and ensuing divisions the same number of the chromosomes is present.

Thus the sporeling from the fertilized gametes of *Cutleria multifida* develops into a structure of the Aglaozonia form of this species, which contains 48 chromosomes.

Aglaozonia reptans

ZOOSPOROGENESIS

The forms which evidently fall under the category of this species show somewhat wide variability in their habit. The mitosis in the vegetative cells of the form was studied. Since the essential features of the division are similar to those of Cutlèria, detailed accounts will be omitted at this time. The fundamental difference between the two forms is that the nucleus of Aglaozonia contains 48 chromosomes, the number persisting up to the formation of the zoosporangium.

Zoosporangia are produced on the upper surface of the thallus. The origin of the structure is as follows: A superficial cell of the thallus slightly elongates and divides, giving rise to two cells, the upper one of which becomes as a rule a zoospore mother cell; not infrequently, however, several cell divisions take place, and in that case the terminal cell becomes the mother cell. The growth of the zoospore mother cell is striking; it elongates until its length becomes three to six times its width. The elongation of the cell is always accompanied by the growth of the nucleus, which remains in the middle region of the cell.

When the nucleus is approaching the prophase, the chromatin network, by a possible rearrangement of the material, becomes less and less branched, and finally there results a tangled mass of continuous threads traversing the nuclear cavity. The tangled threads, becoming more and more uniform in thickness, become transformed into regularly arranged loops centering at a certain part of the cavity, which represents the beginning of the synapsis stage. The synaptic accumulation of the threads occurs always in association with the

kinoplasmic mass outside the nucleus, but its relation to the axis of the cell is varied.

The loops shorten and thicken, and finally they break up into 24 bivalent chromosomes, each derived from one of the loops. The heterotypic figure thus established is intranuclear, and the axis of the spindle is in various directions. Between the first and second divisions the daughter nuclei rest. The four nuclei which are products of the second division contain 24 chromosomes, and the same number is found in the third division which gives rise to eight nuclei.

When the zoospore mother cell has reached the eight-nucleate stage, there occurs generally a cleavage of the cytoplasm, which divides the whole contents of the mother cell into eight zoospore primordia (*Anlagen*). Not infrequently, however, one or two more divisions occur after the third, and as a consequence there are produced 16 or 32 nuclei, and in those cases 16 or 32 zoospores are formed.

The nuclear divisions in the mother cell, as well as the segmentation of the zoospore primordia, always occur simultaneously. As was stated before, the chromosomes contained in the thallus of Aglaozonia are reduced to one-half during the first two divisions in the zoospore mother cell, and 24 chromosomes are involved in the zoospores.

The zoospore germinates independently, without any conjugation; possibly 24 chromosomes, the reduced number, may persist in the structure arising from the germination of the sporelings of the zoospores, but the nuclear details in the sporelings have not yet been completely investigated.

Summary

The nuclear conditions during the life-history of *Cutleria multifida* and *Aglaozonia reptans* may be summarized as follows:

1. The nucleus of both male and female plants of *Cutleria multifida* contains 24 chromosomes; and the male and female gametes produced contain the same number.

2. In the union of gametes the number is doubled, and 48 chromosomes appear in the sporelings, which develop into the Aglaozonia form of Cutleria. Therefore it is evident that the individual bearing the name of *Cutleria multifida* represents the gametophytic phase of

the species, 24 being the gametophytic number of chromosomes; and the Aglaozonia form of Cutleria represents the sporophytic phase of the species, 48 being the sporophytic number.

3. *Aglaozonia reptans* contains 48 chromosomes, and the number is reduced in zoospore formation, the zoospore containing 24 chromosomes. The zoospore with the reduced number of chromosomes germinates without conjugation. Although the nuclear details of the sporelings of *Aglaozonia reptans* have not yet been followed, it seems evident that *Aglaozonia reptans* represents the sporophytic phase of the individual whose gametophytic and sporophytic numbers of chromosomes are respectively 24 and 48. Probably *Aglaozonia reptans* as it occurs in nature is identical with the Aglaozonia form of *Cutleria multifida* which we have grown under culture and is now determined to be the sporophytic phase of the species.

THE UNIVERSITY OF CHICAGO

BRIEFER ARTICLES

OXYGEN PRESSURE AND THE GERMINATION OF XANTHIUM SEEDS

A PRELIMINARY REPORT

Progress in the analysis of the conditions which cause delayed germination in seeds has been disappointingly slow, largely due to the fact that investigators have persisted in neglecting the seed coat as a factor on account of its thinness, as in KINZEL'S recent paper,[1] or because of a preconceived idea that the embryonic protoplasm is more or less dormant in newly ripened seeds, as in FISCHER'S work,[2] and that some stimulus is necessary to activate it.

It has been shown by CROCKER[3] that the seed coat of Xanthium, in spite of its thinness, is the sole cause of delay under normal germinative conditions. He has also shown[4] that the delayed germination in seeds of aquatic plants depends in many instances on the seed coats. It is evident that the testa cannot be neglected as a factor until its insignificance in that rôle has been proven. If seeds are tested with coats removed, it is possible that no dormant protoplasm will be found needing ionic or other stimulus to growth. Investigations in which the seed coat has been arbitrarily neglected for any reason will have to be repeated before a satisfactory interpretation can be attempted.

On the other hand, it is certain that there are cases in which the delay must be attributed to protoplasmic characters, as in Crataegus, the testa of whose seeds has been proven not to be the cause of the delay in germination. The conditions for protoplasmic activity in the embryo of plants have not been analyzed carefully. A series of experiments is being conducted with the seeds of *Xanthium pennsylvanicum* to determine the exact

[1] KINZEL, WILHELM, Lichtkeimung. Einige bestätigende und ergänzende Bemerkungen zu den vorläufigen Mittheilungen von 1907 und 1908. Ber. Deutsch. Bot. Gesells. **26a**:631–645. 1908.

[2] FISCHER, ALFRED, Wasserstoff und Hydroxylionen als Keimungsreize. Ber. Deutsch. Bot. Gesells. **25**:108–122. 1907.

[3] CROCKER, WILLIAM, Rôle of seed coats in delayed germination. BOT. GAZETTE **42**:265–291. 1906.

[4] ———, Germination of seeds of water plants. BOT. GAZETTE **44**:375–380. 1907.

amount of oxgyen necessary for germination with the coats removed. TAKAHASHI[5] has shown that rice germinates in total absence of free oxygen, and CROCKER[6] has shown the same to be true for *Alisma Plantago* and Eichhornia. But the seeds of Xanthium with coats removed remain dormant if oxygen is entirely excluded, though all other germinative conditions are supplied.

The oxygen pressure necessary for the germination of seeds of *X. pennsylvanicum* has been determined with apparatus similar to that used by SCHAIBLE,[7] with modifications to exclude light and to control the temperature. The seeds are soaked in ice water for twelve hours, and the coats carefully removed, thus excluding them as a factor. The temperature was uncontrolled during the first experiments, but it was found immediately that high temperatures would yield results differing from those at low temperatures. The jars, therefore, were kept in a water bath with cold water running through it constantly. The variation in temperature was not more than about two degrees during the time of each experiment. The seeds used at pressures of less than 99^{mm} of mercury were collected in the spring, after lying in the field nearly six months. Those at 99^{mm} and above were collected in the fall as soon as ripe, and were kept in an unheated dry room during the winter and succeeding spring. Each lot of seeds was put on wet absorbent cotton and was subjected to certain conditions of pressure for ten days. The elongation of the hypocotyl, followed by the geotropic response, was used as a criterion of germination.

Since the desired oxygen pressure is secured by a reduction of total atmospheric pressure, the question naturally arises whether the reduction of pressure itself has any influence on the germination. Experiments are being conducted using the same oxygen pressures at full atmospheric pressure to determine whether the mere difference in pressure is a factor. As some time must elapse before these can be continued, I present the results of the first series of experiments in the accompanying table.

The effect of high temperature is seen by comparing the two experiments at 72^{mm}. The experiment at 99^{mm} was conducted with seeds that had been kept in the laboratory over winter, and the temperature averaged nearly 2° lower than the one at 90^{mm}, so that the percentage of germination was slightly less than at 90^{mm}, in spite of the increased oxygen pressure.

[5] TAKAHASHI, T., Is germination possible in absence of air? Bull. Coll. Agr. Tokyo **6**:439–442. 1905.

[6] CROCKER, WILLIAM, Longevity of seeds. BOT. GAZETTE **47**:69–72. 1909.

[7] SCHAIBLE, FR., Physiologische Experimente über das Wachstum und die Keimung einiger Pflanzen unter vermindertem Luftdruck. Beiträge Wiss. Bot. **4**:93–148. 1900.

It is perfectly clear from the figures given that the oxygen pressure necessary for germination is quite low, and that the pressure is not the same for the two seeds. The uppers require a higher pressure than the lowers; this is a real physiologic difference between the two seeds. It must be noticed that the difference in the embryo in the two seeds is in the same direction as the difference in their seed coats, both sets of characters acting in conjunction, not in opposition, in causing a longer delay in the uppers than in the lowers. However, the difference is so slight in the embryonic characters that the germination of the uppers is not at all hindered if the seed coats are off, with full atmospheric pressure. The uppers begin to germinate on the average just a few hours later than the lowers under such conditions.

Atmospheric pressure	Oxygen Pressure	Temperature	Percentage germ. in 10 days				Growth in length of hypocotyl in 10 days (mm)			
			Lowers	Control	Uppers	Control	Lowers	Control	Uppers	Control
99mm	20.72mm	19–22°	75	100	45	95	14.5	30.0	4.9	23.3
90	18.84	21–22.6	80	95	50	100	22.8	45.9	4.3	37.8
*72	15.07	20–28	45	100	20	100	11.5	46.0	9.4	33.6
72	15.07	20–22	30	95	0	100	6.36	28.5	0.0	22.0
*28	5.86	21.5–24.5	0	100	0	95	00.0	37.8	0.0	28.8

*Temperature not controlled.

The seeds which failed to germinate under the experimental conditions of pressure and moisture were in every case brought into normal germinating conditions at the close of each experiment. Germination of 100 per cent. in nearly every instance shows that the seeds are not injured by the experimental conditions.

The surprising feature of the results is the small amount of oxygen pressure necessary for germination. From the rapid exchange of gases which Crocker has shown takes place in the seeds of the cocklebur, one would expect to find a rather high pressure required. The results I have obtained are inconsistent with the rapid respiration which he has shown to occur.

Two things must be taken into consideration in regard to this apparent contradiction of results. In the first place, the seed coats are probably responsible for a large amount of the respiration observed in the seeds of Xanthium with the coats intact. Becquerel[8] has shown that the integuments of seeds produce CO^2 quite freely, often showing a larger output than the seeds from which they are taken.

[8] Becquerel, Paul, Recherches sur la vie latente de graines. Ann. Sci. Nat. Bot. IX. 5: 193–320. 1907.

Moreover, there is a strong correlation between the growth of the hypocotyl and cotyledons. Normally the former grows first, and the latter do not enlarge until the root is well established. But if the seeds with coats on are placed in an atmosphere composed largely of oxygen, this normal correlation is reversed, the cotyledons elongating before the hypocotyl begins to develop. The testa is comparatively thick over the hypocotyl, very thin over the cotyledons, and certainly admits oxygen more quickly to the cotyledons than to the hypocotyl. The cotyledons are less sensitive than the other parts of the embryo, and require more oxygen to activate them than would be necessary for the hypocotyl. It is perfectly clear, then, that much of the oxygen used by seeds which germinate with the seed coats intact and in high oxgyen pressure is due to consumption of oxygen by the seed coats and the cotyledons, very little being used by the hypocotyls. In my experiments the pressure has been determined for the very sensitive hypocotyl, which always grows first if the coat is off, and the pressure required is low. I believe that these two points fully explain the difference in oxygen pressure necessary to germination with coats off and coats intact.

Further work is necessary to determine the exact relation of temperature to the oxygen pressures required, and series at high temperatures will be compared with series at low temperatures to obtain definite data on this point.

Fresh seeds will be collected this fall and tested immediately after they have ripened, to determine whether there is any after-ripening, whether the oxygen pressure necessary for germination is greater or smaller before the period of drying, freezing, and resting than it is later.

Acknowledgments are due to Dr. WILLIAM CROCKER, under whose direction the work here recorded was done.—CHAS. A. SHULL, *Transylvania University, Lexington, Ky.*

CURRENT LITERATURE

BOOK REVIEWS

An American memorial to Darwin

The American Association for the Advancement of Science organized at its Baltimore meeting last Christmas week a worthy celebration of the centennial of DARWIN'S birth and the semicentennial of the publication of the *Origin of species*, part of which consisted of a series of ten addresses by prominent biologists, on topics pertinent to the occasion. These addresses have been published as a memorial volume, under the title *Fifty years of Darwinism*.[1] Of the ten only two are by botanists: "The theory of natural selection from the standpoint of botany," by JOHN M. COULTER, and "The direct influence of environment," by D. T. MACDOUGAL. Yet all will have a definite interest for any botanist who is alive to the questions of evolution.

Professor COULTER, after pointing out the indebtedness of botany to DARWIN for much besides the theory of natural selection, avows that he speaks not for botanists as a whole, but as one "who has had some experience in dealing with facts that enter into phylogenies." This leads him to set forth some of the difficulties encountered, such as the origination of those broad characters that distinguish great groups, and the so-called "adaptations" which prove to be useless or even harmful. The main illustrations are drawn from the gymnosperms, of which group he is a master; and the array is certainly formidable.

Dr. MACDOUGAL discusses the reaction of organisms when subjected to changes in the environment, and the mechanism by which the structural and formal alterations are effected. He cites the recent experiments of RIDDLE, GAGE, TOWER, GAGER, and himself, all of which are well known and have awakened the greatest interest. He believes that GAGER'S experiments and his own indicate that the primary effect is wrought upon the chromosomes of the germ cells; a conclusion that finds support also from GATES'S work on Oenothera and from animal cytology.—C. R. B.

The distinctly zoological addresses are: "Isolation as a factor in organic evolution," by DAVID STARR JORDAN; "The cell in relation to heredity and evolution," by E. B. WILSON; "The behavior of unit characters in heredity," by W. E. CASTLE; "Mutation," by C. B. DAVENPORT; "Adaptation," by CARL

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[1] Fifty years of Darwinism: modern aspects of evolution. Centennial addresses in honor of CHARLES DARWIN before the American Association for the Advancement of Science, Baltimore, Friday, January 1, 1909. 8vo. pp. vi+274. *pls. 5. fig. 1.* New York: Henry Holt & Co. 1909. $2.00.

H. EIGENMANN; "Darwin and paleontology," by H. F. OSBORN; and "Evolution and psychology," by G. STANLEY HALL.

These addresses, by men whose work and writings are concerned with various phases of animal life, deal with problems of wide interest, but in the main they are from the point of view of the zoologist rather than of the botanist, and the material for illustration is largely drawn from animal biology. One gets the impression in reading the different essays that often the point of view of the authors is all too narrow; it is that of the advocate or pleader seeking undue prominence for a certain phase of the evolution problem, rather than that of a man of science considering the phenomena from a broad, impartial point of view.

On the whole, however, the papers are clear, concise, and very much to the point. Due allowance being made for the standpoint from which the individual author took his departure, they present very fairly the average opinions held by the various workers in different fields today. Probably the lay reader will get from the volume what every biologist knows to be true, that the problems of evolution are no longer in the simple state where one factor can be held to explain everything, but that many more factors must be brought in and all harmonized before the evolution question will assume a more unified aspect. He can hardly fail to see clearly that the fact of evolution is beyond dispute, and that it is the method of evolution which is now under discussion.—W. L. TOWER.

Report of American Breeders' Association

The Fifth Annual Report of the American Breeders' Association[2] maintains the high standard which has been shown by all the previous reports of this important organization, and without question contains the most important body of experience and speculation dealing with matters of heredity and breeding as well as of the new science of "eugenics" to be found in America. The present volume is in several respects an improvement over any previous one. A number of useful summaries of breeding work in different crops are given in the form of committee reports, the most important of these dealing with alfalfa, apples, wheat, sugar cane, and tobacco. Special methods for the conduct of practical breeding work in corn, alfalfa, wheat, and sorghum are given. Of over eighty separate articles contained in the volume nearly half have to do with plant breeding, about one-third with animal breeding, and smaller numbers with eugenics, the theory of heredity, and other allied subjects. The most important papers, from a theoretical point of view, are "Some observations in telegony" by E. H. RILEY; "Another mode of species forming," by LUTHER BURBANK; "Some cytological aspects of cotton breeding," by LAWRENCE BALLS; "Characteristics of Wealthy apple seedlings," by W. T. MACOUN; "Clonal or bud-variation," by HERBERT J. WEBBER; "What are 'factors' in Mendelian explanations," by T. H. MORGAN; "Factors for mottling in beans," by R. A. EMERSON; and "The effect of different methods of selection on the fixation of hydrids," by W. J. SPILLMAN. There are

[2] American Breeders' Association. Vol. 5. pp. 420. July 1909. Washington, D. C.

also excellent discussions of work in the production of disease-resistant varieties of flax and wheat by H. L. BOLLEY, and of rice by CHARLES E. CHAMBLISS.

The volume is illustrated with about seventy cuts and diagrams, most of the former being good half-tone engravings. The book is printed on rather cheap paper, but is well bound. It is marred by an undue number of typographical errors, owing to the unfortunate fact that the various papers were not submitted to their authors for correction. The volume closes with a directory of the members of the association, now numbering something over 1200, followed by subject and author indexes to the articles contained in the volume. No biological library can afford to be without these annual volumes, and every one interested in any subject related to heredity or breeding should not fail to become a member of the organization.—GEO. H. SHULL.

Floral biology

Under the general direction of Dr. TOULOUSE, Doin & Fils, Paris, have undertaken the publication of an *Encyclopédie scientifique*. It is divided into 40 sections, each in charge of a special director, and the completed work will comprise about 1000 volumes, each one of which will be a scientific monograph. The classification is exceedingly interesting, botany being represented by three of the 40 sections as follows: 15, *Physiologie et pathologie végétales;* 22, *Botanique;* 35, *Botanique appliquée et agriculture;* not to mention other sections entitled *Biologie*, *Physiologie*, *Pathologie*, etc., which inferentially contain no botany. The section of plant physiology and pathology is under the special direction of L. MANGIN, and is to include 13 monographs, the first one of which to appear is on "Floral biology," by PÉCHOUTRE.[3]

The boundaries of the subject are vague, but after a historical introduction the author presents his material in two parts: (1) Sex and sexual elements, and (2) Pollination and floral structures. The topics of the second part are obvious, and the usual information concerning cleistogamy, dichogamy, etc., is presented as fully as 175 pages will permit, and presumably in a form suited to the prospective audience. Just what may be treated in such a volume under the head of "sex and sexual elements," however, is not so self-evident. In this case the titles of the six chapters are in substance as follows: the separation of sexes in flowering plants; the influence of external agents on the determination of sex in dioecious plants; the phylogeny of the separation of sexes; the transformation of "hermaphrodite" plants into dioecious plants, including "slow variation or mutation;" the sexual elements of "phanerogams," including protection of pollen, formation of gametes, the development of the pollen tube, and fertilization; the dissociation of the vegetative and sexual activities of pollen.

Taking the book as a whole, it is conspicuous for its lack of perspective, perhaps it would be better to say its curious perspective; for its material of very

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[3] PÉCHOUTRE, F., Biologie florale. 12 mo. pp. 369. *figs. 82.* Paris: Octave Doin & Fils. 1909. 5*fr.*

unequal values, assorted in such a way as to indicate information that comes from reading rather than knowledge that comes from investigation; and for its evidence of a very limited acquaintance with the modern literature, including as it does some of the best literature, dealing with the subjects presented. The desire to interpret science to the reading public is a worthy motive, and it ought to appeal more strongly to men of science than it does; but the interpretation must represent current science, or it will deceive rather than inform.—J. M. C.

MINOR NOTICES

Das Pflanzenreich.[4]—Part 38 of this work consists of a monographic treatment of the Cyperaceae-Caricoideae by the distinguished caricologist Professor GEORG KÜKENTHAL. The author follows the usual sequence of this excellent series of monographs in the general consideration of the group. Four genera are included, namely, Schoenoxiphium (6 species), Cobresia (29 species), Uncinia (24 species), and Carex (793 species). The total number of species representing the four genera, as here treated, includes only about a dozen which are characterized as new, and of the new species not one is recorded from America. Several new American varieties, however, are described. The nomenclatorial changes are relatively few. The chief interest of the publication centers on the genus Carex, which is divided into four subgenera and fourteen sections; the divisions are based primarily on the characters of the inflorescence. The keys preceding the species of each section are concise and well contrasted, the descriptions are carefully drawn, the literature and exsiccatae are freely cited, and the illustrations are numerous and well selected. On the whole the present monograph should materially aid toward a better understanding of this difficult but interesting genus.

Part 39 contains an elaboration of the Phytolaccaceae by DR. HANS WALTER. The author recognizes for this family 24 genera and 114 species, of which 32 are new to science. In addition to the general index there is a list of the collectors mentioned and numbers cited in the body of the work, which facilitates greatly the organizing of herbarium material in accordance with the text.—J. M. GREENMAN.

Chronology of the flora of Italy.[5]—The present volume is an analysis of the flora of Italy with particular reference to its historical development. The main body of the work is essentially a catalogue of the species, including those indigenous, introduced, and naturalized, also those in cultivation. The sequence of the genera is in accordance with ENGLER and PRANTL'S *Natürlichen Pflanzenfamilien*. About 4100 species, many varieties, and hybrids are listed, and under the species reference

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4 ENGLER, A., Das Pflanzenreich. Heft 38 (IV. 20). Cyperaceae-Caricoideae von GEORG KÜKENTHAL, pp. 824. *figs. 128 (981)*. *M* 41.20. Heft 39 (IV. 83). Phytolaccaceae von HANS WALTER, pp. 154. *figs. 42 (286)*. *M* 7.80. Leipzig: Wilhelm Engelmann. 1909.

5 SACCARDO, P. A., Chronologia della flora Italiana. Royal 8vo. pp. xxxvii+390. Padova: Tipografia del Seminario. 1909. L.15 ($2.90).

is made to the first record of the plant in Italian literature, as well as subsequent mention by later writers. This volume is the result of much painstaking labor, and it presents a mass of historical information in epitomized form. An excellent bibliographical catalogue adds materially to the value of the publication as a work of reference.—J. M. GREENMAN.

NOTES FOR STUDENTS

Current taxonomic literature.—H. DE BOISSIEU (Bull. Soc. Bot. Fr. IV. 9:348–355. 1909) describes several new species and varieties of Umbelliferae from China, and proposes a new genus (*Chaerophyllopsis*) of this family, which is referred to the tribe Ammineae.—J. D. HOOKER (Kew Bull. 1909: 281–289) in a "Review of the known Philippine Islands species of Impatiens" recognizes 25 species and precedes their enumeration by a determinative key.—C. H. WRIGHT (*ibid.* 308) has published a new genus (*Neodregea*) of the Liliaceae from South Africa.—F. J. SEAVER (Mycologia 1:177–207. *pl. 13.* 1909) under the title "The Hypocreales of North America II" gives a systematic treatment of the tribe Creonectrieae, recognizing 11 genera to which are definitely referred 38 species; 11 additional species are mentioned as of doubtful generic affinity. Five of the genera (*Sphaerodermatella, Creonectria, Macbridella, Scoleconectria,* and *Thyronectroidea*) are new, and of the 38 species 29 form new combinations.—F. D. KERN (*ibid.* 208–210) records a new species of Gymnosporangium from Colorado.—F. D. HEALD (*ibid.* 215–217. *pl. 14*) describes and illustrates a new species of Discosia parasitic on pine seedlings at Halsey, Nebraska.—F. OSTERMEYER (Ann. K. K. Naturhist. Hofmus. 22:128–142. 1907–1908) publishes a list of about 300 plants collected in Brazil in 1860 by Dr. THEO. PECKHOLT; the list contains, among other novelties, a new species of Cryptocarya.—F. KRÄNZLIN (*ibid.* 1911–1996. *pls. 3, 4*) under the title "Beiträge zur Kentniss der Gattung Calceolaria" has published new species of this genus from Central and South America.—A. BRAND (Philip. Jour. Sci. 4:107–110. 1909) has described 5 new species of Symplocos from the Philippine Islands.—E. B. COPELAND (*ibid.* 111–115) in continuation of his studies on Philippine ferns records 7 new species and proposes one new genus (*Curriania*).—E. D. MERRILL (*ibid.* 117–128) presents a "Revision of the Philippine Connaraceae," recognizing 5 genera and 17 species of which 8 are described as new; the same author (*ibid.* 129–153) under the title "A revision of Philippine Loranthaceae" recognizes 6 genera and 53 species of which 19 are new; one new genus (*Cleistoloranthus*) is proposed.—H. N. RIDLEY (*ibid.* 155–199) gives a synopsis of the Scitamineae of the Philippine Islands. The group includes four families, as follows: Zingiberaceae with 15 genera and 61 species, Marantaceae with 4 genera and 7 species, Cannaceae with 1 genus and 2 species, and Musaceae with 1 genus represented by 1 endemic and 4 cultivated species. Several species are here described for the first time.—A. DECANDOLLE (Leafl. Phil. Bot. 2:633–638. 1909) gives a "Revision of the Philippine species of Elaeocarpus," in which 16 species are recognized, 3 being new.—A. ENGLER (Bot. Jahrb. 43:303–381. 1909), in cooperation with several botanists, under the title

"Beiträge zur Flora von Afrika XXXV" has published 125 new species and several varieties of flowering plants. The following new genera are proposed: *Lingelsheimia*, *Baccaureopsis*, and *Milbraedia* of the Euphorbiaceae, *Pierrina* of the Scytopetalaceae, and *Ledermanniella* of the Podostemonaceae. The contribution is based chiefly on the collections of Dr. J. MILBRAED.—O. MÜLLER (*ibid.* Beibl. No. 100. pp. 1–40. *pls. 1, 2*) lists a large number of Bacillariaceae from southern Patagonia from the collections of E. NORDENSKIÖLD and O. BORGE. Several new species and varieties are described.—E. L. GREENE (Rep. Nov. Sp. 7: 195–197. 1909) under the title "Novitates Boreali-Americanae IV" has published 7 new species of sympetalous plants.—J. R. DRUMMOND (Curtis' Bot. Mag. IV. 5: *t. 8271.* 1909) describes and illustrates a new species of Agave from Central America. M. GURKE (Monats. Kakteenk. 19: 116–121. 1909) describes and figures a new species of cactus (*Cephalocereus DeLaetii*) indigenous to Mexico.—W. FAWCETT and A. B. RENDLE (Jour. Bot. 47: 263–266. 1909) in continuation of their studies on Jamaican orchids have published 6 new species and include 1 new genus (*Harrisella*) which is based on *Aeranthus porrectus* Reichb.—A. and E. S. GEPP (*ibid.* 268, 269) have described a new species of Udotea from St. Thomas.—W. A. MURRILL (Mycologia 1: 140–160. 1909) in a second article on the "Boletaceae of North America" gives a synopsis of the genus Ceriomyces, recognizing 35 species, and (*ibid.* 218, 219) describes a new species of this genus from the volcano of Turrialba, Costa Rica.—E. ROSENSTOCK (Rep. Nov. Sp. 7: 146–150. 1909) under the title "Filices Novae V" describes new species of ferns, 3 of which are from Ecuador.—F. C. CLEMENTS and H. L. SHANTZ (Minn. Bot. Studies 4: 133–135. *pl. 20.* 1909) have proposed a new genus (*Eucapsis*) of the blue green algae; the genus is represented at present by a single known species (*E. alpina*) from Colorado.—C. H. PECK (Bull. Torr. Bot. Club 36: 153–157. 1909) has published 10 new species of North American fungi.—J. K. SMALL (*ibid.* 159–164) in an article entitled "Additions to the flora of peninsular Florida" records several species hitherto unknown from the mainland and describes 5 new species.—A. D. E. ELMER (Leafl. Philip. Bot. 2: 595–629. 1909) has described 11 species and 2 varieties of Philippine plants as new to science. Synopses of the Philippine species of Fagraea, Artocarpus, and Hydrocotyle are given, and a new generic name (*Adelmeria* Ridl.) is proposed to take the place of Elmeria recently described in this journal.—J. M. GREENMAN.

Corn breeding.—Several recent papers have appeared advocating the use of hybridization methods in the production of Indian corn, instead of the usual ear-to-the-row method which is based upon the idea of isolation of pure types. As early as 1893 and 1894 GARDNER and MORROW[6] showed that crosses between different strains of corn give somewhat increased yields over either of the parent strains, and a method was outlined by which this advantageous circumstance could

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[6] MORROW, G. E., AND GARDNER, F. D., Bulletins 25 and 31, Illinois Agricultural Experiment Station. 1893, 1894.

be readily utilized. Two years ago the reviewer read a paper[7] before the American Breeders' Association in which it was shown that a field of Indian corn consists of a large number of elementary species thoroughly hybridized in complex fashion, and gave evidence that the vigor necessary to the production of large yields is due to the degree of heterozygosis possessed by the individuals composing the crop. This paper closed with the suggestion that "continuous hybridization instead of the isolation of pure strains is perhaps the proper aim of the corn breeder." Based upon this conception the reviewer[8] worked out a scheme of corn breeding in which definite pure lines were isolated and recombined, so that the field crop would consist of first-generation hybrids between these two pure lines, thus insuring perfect uniformity as well as high yield. Simultaneously with this latter paper there appeared two other papers presenting suggestions for a similar method of corn breeding. In the first of these EAST[9] suggests the purchase by the farmer of two highly bred strains from the professional corn breeder, and the hybridization of these two strains each year to produce the seed corn for the field crop, arguing that the methods used by the professional breeder are such as to render these strains already to a considerable extent homozygous. This method is the same as that of MORROW and GARDNER. EAST recognizes the relation between this method and the pure-line method of the reviewer, saying that the latter is more correct theoretically, but less practicable than the method he suggests. EAST gives a clear and incisive discussion of the significance of the pure-line idea. COLLINS[10] has issued a bulletin also advocating the use of continuous hybridization in corn breeding. The appearance of three papers simultaneously advocating the same innovation in corn breeding is likely to have great influence on the activity of those engaged in this work. The bulletin by COLLINS is unfortunately quite vague in its language, as might be inferred from the title, "The importance of broad breeding in corn." Comparing the conception of "broad breeding" with the conception involved in the other two papers appearing simultaneously with it, both of which are expressed in terms of definite hybridization, gives a fair indication of the relation between these papers. In keeping with his title, COLLINS says "had it been realized that diversity is as necessary to the life of the species as is chlorophyll to the life of the individual, it would have been evident that one might as well breed to eliminate the green color from the leaves as to suppress this corn variation." He says also that "the appearance of so-called barren stalks in a field of corn may be thought of as an adaptation to avoid self-pollination," and adds that "the elimination of these

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[7] SHULL, G. H., The composition of a field of maize. Amer. Breeders' Assoc. **4**:296–301. 1908.

[8] ———, A pure line method in corn-breeding. Amer. Breeders' Assoc. **5**:51–59. 1909.

[9] EAST, E. M., The distinction between development and heredity in in-breeding. Am. Nat. **43**:173–181. 1909.

[10] COLLINS, G. N., The importance of broad breeding in corn. U. S. Bureau Plant Industry, Bull. 141[4]:33, 34. 1909.

proterandrous plants results in increasing the percentage of self-pollinated plants, and is a practice of doubtful value."

SMITH[11] has issued a bulletin dealing primarily with the results of selection of ears which are placed high on the stalks and those which are placed low on the stalks, and showing that very material difference may be secured by five years' selection. A comparison is then made between the two strains so produced in regard to qualities such as time of maturity, yield, etc. The results accord very well with the notion that ordinary varieties of corn are much hybridized, and that the selection results in a partial separation of the biotypes involved.—GEO. H. SHULL.

Morphology and sexuality of Aspergillus and Ascophanus.—In *Aspergillus repens*, a form differing slightly in structure from *Aspergillus herbariorum* as described by Miss FRASER and Miss CHAMBERS,[12] Miss DALE[13] describes another case of so-called reduced fertilization among the Ascomycetes. After a brief historical and systematic consideration of the species, she describes the multinucleate hyphae of the mycelium, from which arise the multinucleate conidia as apical swellings. The archicarp is initiated as a slender branch, which usually soon becomes regularly and closely coiled into a spiral. The regular occurrence of definite ascogonia and antheridia as figured by DEBARY was rarely found, the antheridium often being absent. No convincing proof of a fusion of sexual organs, even when both were present, was discovered. Transverse walls, whose position and number vary considerably, appear in the archicarp either very early or at a much later stage. Ascogenous hyphae develop in some cases from all of the cells of the ascogonium. The investing hyphae show great variations in the time at which they arise, as well-developed ascogonia with ascogenous hyphae quite uninvested are often found. The young archicarp, which arises as a multinucleate branch, possesses nuclei of about uniform size. Later variations in size of the nuclei appear, which Miss DALE accounts for chiefly by a fusion in pairs, although nuclei may perhaps grow. Such nuclear fusions are figured in all cells of the ascogonium. Since no antheridium is believed to fuse with the oogonium, these nuclear fusions are held to be reduced sexual ones. These fusion nuclei pass into the ascogenous hyphae. In the development of the ascus, which arises from the penultimate cell of a hypha, the usual nuclear fusions and subsequent triple divisions occur. Karyokinesis was not observed.

CUTTING[14] finds in *Ascophanus carneus* still another case of reduced fertiliza-

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[11] SMITH, L. H., The effect of selection upon certain physical characters in the corn plant. Ill. Agric. Exper. Sta. Bull. 132:50–60. 1909.

[12] FRASER, MISS H. C. I., AND CHAMBERS, MISS H. S., The morphology of *Aspergillus herbariorum*. Ann. Mycol. **5**:419–431. 1907.

[13] DALE, MISS E., On the morphology and cytology of *Aspergillus repens* DeBary. Ann. Mycol. **7**:215–225. 1909.

[14] CUTTING, E. M., On the sexuality and development of the ascocarp of *Ascophanus carneus* Pers. Annals of Botany **23**:399–417. 1909.

tion. The fungus, whose spores start to germinate in alkaline media, was not successfully cultivated in the laboratory. The cross-walls of the multinucleate cells of the vegetative hyphae have a pore, on each side of which are a number of deeply staining granules, whose function was not determined. The archicarp, a scolecite arising as a branch from a vegetative hypha, consists of a basal vegetative portion, a central ascogonial part, and a terminal vegetative region, which is regarded as a functionless trichogyne. The number of cells in each of these regions varies greatly. From the basal region numerous investing hyphae arise. Although the ascogonia grow crowded together, each fruit arises from a separate ascogonium. The cells of the ascogonial portion have each a pore in their transverse walls, which is guarded by small granules early fusing together to form a pad closing the pore. This pad eventually disappears, leaving the multinucleate ascogonial cells in communication. Nuclear fusions are believed to occur in all of the cells of the ascogonial portion. No nuclear migrations were observed, fusions occurring even before the pads disappear. Ascogenous hyphae arise from any or all of the ascogonial cells. Each ascogonial cell is regarded as female, and in the absence of an antheridium the nuclear fusions are held to represent a type of reduced fertilization. The asci develop from the penultimate cells of the recurved tips of the ascogenous hyphae in the usual way. The usual nuclear fusions and divisions occur in the development of the ascus. No karyokinetic figures were observed, and the method of spore-formation is not described.—J. B. OVERTON.

Respiration and fermentation.—KOSTYTSCHEW, in a preliminary paper,[15] points out that "the metabolism of the complex processes of vital oxidation remain yet quite unexplained, chiefly because the oxidases, according to the latest researches, are unable to produce a direct combustion of the carbohydrates." Conceding that anaerobic respiration is identical with alcoholic fermentation, he enumerates the possibilities as to the rôle of zymase in respiration: (1) the zymase of seed plants is not identical with that of yeast; (2) alcoholic fermentation in seed plants occurs in the presence of O_2, but has nothing to do with aerobic respiration; (3) alcoholic fermentation is the first stage of aerobic respiration, the alcohol formed being oxidized to CO_2 and H_2O; (4) alcoholic fermentation is the first stage of aerobic respiration, but in the presence of air under normal conditions no alcohol is formed, because the intermediate products are oxidized; (5) alcoholic fermentation is the first stage of aerobic respiration, but the alcohol is used as constructive material. Of these possibilities he eliminates several, citing various researches which bear on them, and reports his own investigations, which indicate the correctness of the fourth hypothesis above. The small quantities of alcohol that have been observed by some investigators are easily accounted for by the assumption that the oxidative power of the plant does not always keep exact pace

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[5] KOSTYTSCHEW, S., Ueber den Zusammenhang der Sauerstoffatmung mit der Alkoholgärung. Ber. Deutsch. Bot. Gesells. **26**:565–573. 1908.

with the formation of primary cleavage products, in which case these are elaborated into alcohol and CO_2.—C. R. B.

A new fossil araucarian.—SINNOTT[16] has described a new genus of araucarian wood from the Cretaceous (?) clays of Scituate, Mass. The structure of *Paracedroxylon scituatense* consists of tracheids and pith rays, the radial pits of the former being circular (not flattened by mutual contact), and the cells of the latter thin-walled and without pits except in the walls adjacent to the tracheids. Groups of thin-walled cells which occur in wounded regions are thought possibly to represent abortive resin canals, and in the bands of wound tissue which occur near wounds large anastomosing mucilage spaces appear which are said to represent somewhat modified traumatic resin canals. The conclusion is that Paracedroxylon is another primitive araucarian which is "on the border line between this group and their ancestors, the primitive Abietineae," which "probably left the ascending araucarian line before the appearance of flattened pitting," and whose "traumatic canals were subsequently much reduced from the typical abietineous condition."—J. M. C.

Oospheres of Sargassum.—In a short preliminary note, TAHARA[17] announces the periodic liberation of the oospheres of the species of Sargassum (about 10 in number) at the Misaki Marine Biological Station of the Tokyo Imperial University, *S. enerve* being the species chiefly under observation. The liberation occurs simultaneously not only in a given individual, but also in all the plants of the locality, proceeding in fortnightly crops on a particular day, at a fixed interval after the highest spring tide, this interval varying in different species. All the oospheres of a single conceptacle are not discharged at one time, but in two or three successive fortnightly crops.—J. M. C.

Light and protein synthesis.—ZALESKI, after further experiments,[18] has supported the general view that light promotes the synthesis of proteins only indirectly, because of the relation to the synthesis of carbohydrates. He rejects the researches of LAURENT and of GODLEWSKI as insufficient to show the direct influence of light upon any molecular combination in the proteins.—C. R. B.

[16] SINNOTT, EDMUND A. W., Paracedroxylon, a new type of araucarian wood. Rhodora **11**:165–173. *pls. 80, 81.* 1909.

[17] TAHARA, M., On the periodical liberation of the oospheres in Sargassum. Preliminary note. Bot. Mag. Tokyo **23**:151–153. 1909.

[18] ZALESKI, W., Ueber dei Rolle des Lichtes bei der Eiweissbildung in den Pflanzen. Ber. Deutsch. Bot. Gesells. **27**:56–62. 1909.

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THE BOTANICAL GAZETTE

December 1909

Editors: JOHN M. COULTER and CHARLES R. BARNES

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BOTANICAL GAZETTE

DECEMBER 1909

DIOON SPINULOSUM [1]

CHARLES J. CHAMBERLAIN

(WITH SEVEN FIGURES)

Three well-defined species of Dioon have been found in the Mexican tropics: *D. edule* Lindl., *D. spinulosum* Dyer, and *D. Purpusii* Rose. *D. edule*, which was described as early as 1843, is now quite well known. Although *D. Purpusii* was described only a few months ago, the species had often been seen, but had been mistaken for *D. edule*, which it resembles in its general appearance. I was told by a Mexican botanist that I should find *D. edule* along the Mexican Southern R. R. in the neighborhood of Santa Catarina, State of Oaxaca. He said there was no other cycad in that region. The plant was easily found, and I at once saw that it was a new species intermediate between *D. spinulosum* and *D. edule*. The plant called *D. edule* in *fig. 101* of WIELAND'S *American fossil cycads* is the new *D. Purpusii*, as plainly indicated by the staminate cone and by the leaves. MACDOUGAL and ROSE collected cones of the new species in 1906 in the Tomellin cañon, where it was well shaded by bushes and small trees. PURPUS in 1908 collected seeds and bracts in the Sierra Mixteca, Puebla. In April of the same year I saw the species at various places between Santa Catarina and Tomellin, growing in dry, exposed situations, associated with cacti and Beaucarnea. ROSE[2] describes the staminate sporophylls as "bracts with recurved ovate tips," apparently supposing that the sporangia were borne on the

Investigation prosecuted with the aid of a grant from the Botanical Society of America.

[2] ROSE, J. N., Studies of Mexican and Central American plants. Contrib. U. S. Nat. Herb. **12**:259–302. 1909.

adaxial surface, but of course they are on the under surface and the tips point up instead of down. His description of "seeds about 4^{cm} in diameter" needs confirmation; 4^{cm} in length would be more probable.

In general habit, in the straight, stiff, ascending leaves, *D. Purpusii* resembles *D. edule,* and the leaflets have the texture of those of *D. edule,* but they retain in some degree the spinulose character of those of *D. spinulosum.* In the number of sporangia on a microsporophyll, *D. Purpusii* is intermediate between *D. spinulosum* and *D. edule.* The ovulate sporophyll is rather slender and tapers gradually to a point, in this feature resembling *D. edule* and differing decidedly from *D. spinulosum.*

Dioon spinulosum Dyer was described by EICHLER[3] in 1883, the description being based upon a few leaves and a few trunks without leaves. Since the largest leaf was only 65^{cm} in length, the plants must have been very small. One of the trunks received by EICHLER produced a leaf which his figure shows to be that of a plant only a few years old. The material came from a nursery in Cordoba, State of Vera Cruz, Mexico, and, according to the gardener, the plants grew wild in the neighborhood of Tuxtla. The striking feature is the spinulose leaf. No cones were found, and so EICHLER remarks that it might seem doubtful whether the plant is really a Dioon ("Es möchte daher zweifelhaft erscheinen, ob die Pflanze wirklich ein Dioon ist").

DYER obtained a leaf from Yucatan, and since the locality was given as Progreso, the leaf probably came from a cultivated specimen. EICHLER agreed that DYER should describe the new species. The description, based upon the single leaf, is as follows:[4]

Folia breviter petiolata, elongato-lanceolata, rigida, plana, pinnatisecta, ad 3 pedes longa; segmentis circiter 70 utroque latere, mediis majoribus subopppositis lineari-lanceolatis breviter acuminatis 18–23-nerviis, ad 4 pollices longis media latitudine semipollicaribus, basi angustiore, utroque latere spinulis pungentibus basim versus integerrimis, inferioribus in dentes palmatifidos desinentibus. *Strobili.* . . . . South Mexico, Tuxtla; Yucatan, Progreso. (C. J. Hoge). Herb. Kew.

[3] EICHLER, A. W., Ein neues Dioon. Gartenflora **2**:411–413. 1883.

[4] DYER, W. T. THISTLETON, Cycadaceae, in Biologia Centrali-Americana Botany **3**:190–195. 1882–1886.

During my second trip to Mexico, in 1906, I saw small specimens of *Dioon spinulosum* in the park at Vera Cruz, but did not find it growing wild. Gov. Teodore A. Dehesa, of the State of Vera Cruz, who has repeatedly assisted me in my investigation of Mexican cycads, again used his influence in my behalf, and I am also under renewed obligations to Mr. Alexander M. Gaw, of the State Bureau of Information, Jalapa, Mexico, for his continued interest and active cooperation both in securing material and often in furnishing field notes.

Mr. Gaw found that the plants in the park at Vera Cruz came from Tlacotalpam, a town southeast of Vera Cruz, and he also found that most people do not distinguish the two species, *D. edule* and *D. spinulosum*, for he was told that the plants in the park at Vera Cruz could be found growing wild in the vicinity of Tlacotalpam, Catemaco, and elsewhere in the canton of Tuxtlas, and between Palmar and Colorado on the Interoceanic R. R. Doubtless the information in regard to the first three places was correct, but since I had found only *D. edule* in the Palmar-Colorado region, Mr. Gaw sent a man through that entire district with a leaf of *D. spinulosum* as a guide. The man found *D. edule* in abundance, but failed to find a single specimen of *D. spinulosum*. In Vera Cruz the plant is called *palma de Dolores*, the same name which in the Jalapa region is applied to *D. edule*. The name *tio tamal*, which is commonly given to *D. edule* because the seeds are used in making *tamales*, does not seem to be applied to *D. spinulosum*, although its seeds are edible. The natives of the coast region call both the plant and the seeds *chicalitos*, a name which I have not heard applied to *D. edule*.

From a park in Tlacotalpam Mr. Gaw secured a large ovulate cone of *D. spinulosum*, and he was told that the specimen came from the Sierra de Oaxaca Mountains near Tuxtepec, where the plant was said to be very abundant. As the Tlacotalpam cone had only abortive seeds, Mr. Gaw, after repeated efforts, succeeded in securing from the Tuxtepec region an ovulate cone in which pollination and fertilization had taken place. Later he secured a cone with ripe seeds, which germinated readily. With the material Mr. Gaw sent the rather startling information that, according to the natives, the cones are borne below the crown of leaves and not above the crown, as in *D. edule*.

In March 1908 I visited southern Mexico to collect *D. spinulosum*. While on the way to Tuxtepec, I was informed that a plant which seemed to agree with my description, except that it was much taller, could be found near Tierra Blanca. In the mountains west and a little north of Tierra Blanca I found a few specimens and was told that plants were more numerous a few miles farther south. The information was correct, for on the immense hacienda of the Joliet Tropical Plantation Company, a short distance from Tierra Blanca and about 60 miles south of Vera Cruz, magnificent specimens are abundant. Mr. J. C. DENNIS, superintendent of the plantation, very generously furnished horses, guides, and the hospitality of his palatial home while I explored the mountains and secured photographs and material. The plant is usually well shaded, growing among the prevailing limestone rocks which have given name (Tierra Blanca) to the region.

From Tuxtepec, a town on the Papaloapam River about forty miles southwest of Tierra Blanca, half a day's ride on horseback brings one to the mountains where *D. spinulosum* is as abundant as at Tierra Blanca. In some places it is the only large plant, and it would not be an exaggeration to speak of a Dioon forest. Beautiful specimens, which might have been the pride of any conservatory, had been cut down to get the cones, because it was easier to cut the tree than to climb it. The natives use the young seeds in making *tamales*, as in *D. edule*, and at a later stage the dry stony seed coat is a common plaything for children. At Tuxtepec the dry seeds, each pierced with two holes, sell for fifteen cents a dozen. The Indians said that the plant extends some distance farther south, but that it does not occur on the western (Oaxaca) side of the mountains.

Dioon spinulosum, with the exception of the Australian *Cycas media*, is the tallest cycad known, and its slender trunk with a large crown of leaves gives it the appearance of a palm (*fig. 1*). I measured specimens 12^m in height, and Drs. BARNES and LAND, visiting the Tierra Blanca region a few months after my return, found specimens more than 16^m in height, almost as high as the tallest known specimens of *Cycas media*. The slender trunk and graceful curve of leaves are in striking contrast with the stocky trunk and straight, rigid, ascending leaves of *D. edule*.

FIG. 1.—*Dioon spinulosum* on the Hacienda de Joliet near Tierra Blanca, March 1908; the tallest plant is about 10^{m} in height.

The ribbed surface of the trunk is due to successive crowns, and affords some basis for an estimate of the age of the plant, but in the lower part of the trunk of tall specimens the ribs may become too obscure for accurate counting. The scars of the individual leaves, which are so distinct in *D. edule*, are so obscure in *D. spinulosum* that counting in the lower portion of large trunks is out of the question. Whether the duration of the crown is two years, as in *D. edule*, I was not able to determine, but assuming the duration to be two years, the tallest specimens might not be more than 400 years old. However, any estimate can be little more than a guess, until the duration of crowns, and the effect of scale leaves, cones, and resting periods upon the growth of the trunk have been studied much more carefully. Still, I am inclined to believe that a 10^{m} specimen is no older than a 1^{m} specimen of *D. edule*.

At the base the trunk is much enlarged, often having twice as great a diameter as it has 0.5^{m} higher up. The roots are often exposed for considerable distances, especially in precipitous places, where a root may hang down along the surface of the rock. One root hanging down in this way was exposed for a distance of more than 12^{m} and was 5^{cm} in diameter where it again entered the soil.

Cutting through the trunk, which is not nearly so hard as that of *D. edule*, it is seen that there is a single zone of wood surrounding the large pith. The origin and development of the vascular cylinder, as seen in embryos and young seedlings, is being studied by HELEN A. DORETY.

Both EICHLER and DYER gave accurate descriptions of the leaves as they appear on small plants. EICHLER'S largest leaf (65^{cm} in length) had 38 pinnae, the lowest of which were rudimentary. DYER'S leaf was about 1^{m} long, with 70 pinnae on each side. On plants 2^{m} or more in height the leaves reach their full size, so that leaves 2^{m} long, with 100 leaflets on each side, are not uncommon, while one leaf measured 2.2^{m} in length and had 117 leaflets on each side, the leaflets being 20^{cm} long and 10^{mm} wide. There is great variation in the leaflets, some only $14–15^{cm}$ long being 15^{mm} wide. There are 5–8 sharp spines on each margin of the leaflets of large leaves. The lower leaflets become more and more reduced, until the lowest ones are nothing but spines, so that the leaf bears considerable resemblance

to that of Encephalartos. The leaves of seedlings have comparatively few leaflets, the first leaf sometimes having less than a dozen on each side. It is interesting to note that in seedlings the leaves have no reduced leaflets, even the lowest being as perfectly formed as those in the middle of the leaf. The leaflets of seedlings have fewer spines than those of older plants, there being only 2–6 on each margin. The midrib is not so large as in *D. edule* and the leaves are much thinner. A few leaflets of a leaf of medium size are shown in *fig. 2*.

Remembering that the natives had reported cones growing below the crowns, one would naturally think of the condition in Bennettitales.

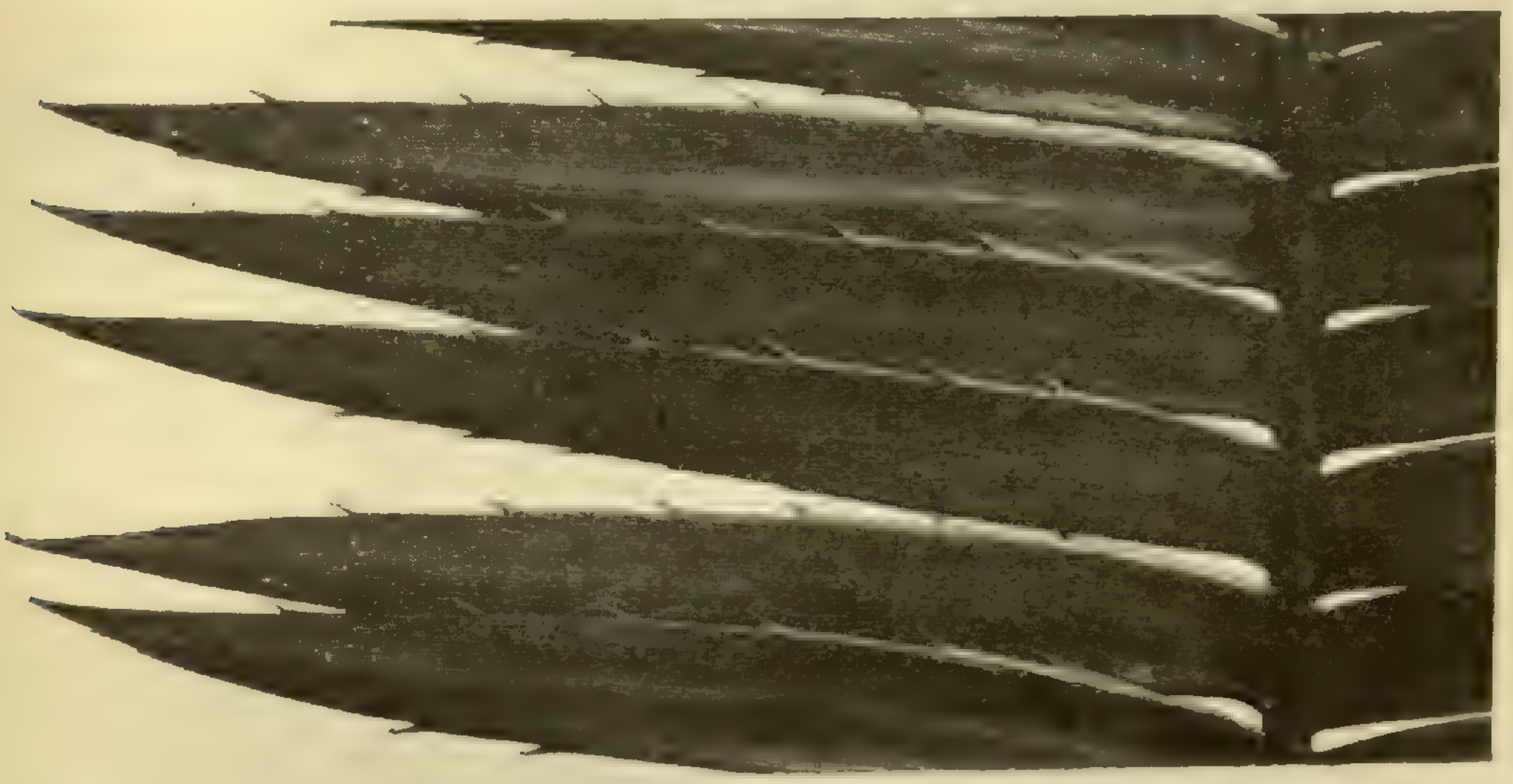


FIG. 2.—Portion of leaf of *Dioon spinulosum* from a specimen at Tlacotalpam; $\times\frac{3}{4}$; the cone shown in *fig. 4* came from the same plant.

A glance at *fig. 3* will show that the cone does hang down below the crown. An examination of the apex of the stem shows, however, that the cone is borne in the center of the crown as in *D. edule*, but that a considerable elongation of the peduncle, together with the great weight of the cone, causes the cone to bend over, slip between the leaves, and thus hang below the crown. Consequently no seeds are found in the nest of the crown as is so commonly the case in *D. edule*, where the germination of seeds in this position often gives rise to the appearance of branching. In *D. spinulosum* branching is rare. Seedlings are found for a considerable distance around the large ovulate plants. The natives say that at maturity the cone bursts with a loud noise, scattering the seeds, or *coyoles*, the plant being called *coyolillo*.

FIG. 3.—*Dioon spinulosum* with ovulate cone, on the Hacienda de Joliet, March 1908.

The ovulate cone is the largest yet known for any gymnosperm. In March, cones weighing 14 kilos were not infrequent, and occasionally a cone had reached a weight of 15 kilos. Since the seeds are not fully mature until October, it is safe to assume that the cones increase somewhat in weight after March, the time of my visit. The cone is cylindrical ovoid (*fig. 4*), its general habit distinguishing it at once from the ovoid cone of *D. edule*. It reaches a length of 50^{cm} and a diameter of 27^{cm}, but the average cone is about 20 per cent. smaller than these measurements.

FIG. 4.—*Dioon spinulosum;* ovulate cone from a cultivated plant at Tlacotalpam; $\times\frac{1}{3}$; November 1906.

The ovulate sporophylls are very hairy, as in *D. edule* and *D. Purpusii*, but are much more closely imbricated, there being no projecting tips as is always the case in the upper part of the cone of *D. edule* and probably in *D. Purpusii*. In regard to the latter species, however, my statement is based upon only a single sporophyll. The

sporophyll of *D. spinulosum* is thick, fleshy, and rounded or obtuse at the apex, contrasting again with the long tapering sporophylls of *D. edule* and *D. Purpusii*. The contour of the exposed portion of the sporophylls and the close imbrication is seen in *fig. 4*, especially near the apex of the cone.

The seeds are white and perfectly smooth, but may become slightly yellowish when mature; their length varies from 4 to 5.5^{cm} and the diameter from 2.5 to 3.5^{cm}. Some of the ovules have the

FIG. 5.—*Dioon spinulosum;* dorsal view of an ordinary sporophyll, the ovule on the left showing the false stalk; from a cone received from Tuxtepec, April 1907. ×¾.

FIG. 6.—*Dioon spinulosum;* ovulate sporophyll, from the cone shown in *fig. 4*, with five ovules, three of which can be seen on the left side, but only a portion of one of the two on the right side is visible. ×¾.

false stalk, characteristic of the genus, but it is not as frequent as in *D. edule* (*fig. 5*). In one cone, with only abortive ovules, there were frequently more than two ovules on a sporophyll, in some cases as many as five or six (*fig. 6*). Cycas, the most ancient genus of the family, regularly produces more than two ovules on a sporophyll, and in Dioon the production of more than two ovules is doubtless a recurrence of the ancient habit. In rare cases, I have noted as many as four ovules on the sporophylls of *Zamia floridana* and *Ceratozamia mexicana*.

As yet I have obtained only one staminate cone, and that not from the field but from Professor TRELEASE, of the Missouri Botanical Garden. Where the specimen came from could not be determined, except that it had been secured from the nursery of W. A. MANDA (S. Orange, N. J.), who had gotten it in a miscellaneous collection of unknown sources. This cone, which arrived in Chicago July 25, 1907, measured 21^{cm} in length by 10^{cm} in diameter, and since the pollen was nearly mature, this must be about the size before the elongation of the axis begins to separate the sporophylls and liberate the pollen. The general appearance of the cone is shown in *fig. 7*.

FIG. 7.—*Dioon spinulosum;* staminate cone from a plant in the Missouri Botanical Garden, July 1907. $\times\frac{2}{3}$.

The shape of the microsporophyll and its general appearance is about as in *D. edule*, except that the microsporangia are much more numerous, the average number on a microsporophyll being about 750; while in *D. edule* the average falls a little below 300. The number in *D. Purpusii* is between 300 and 400. In all three species the sporangia are in sori of 3, 4, 5, and 6 sporangia, with 4 and 5 the most frequent numbers.

While it is hardly safe to arrange the three species in an evolutionary sequence before the life histories have been studied, it seems to me that *D. spinulosum* is the oldest and *D. edule* the most recent, so that the series should be *D. spinulosum*, *D. Purpusii*, and *D. edule*. In favor of this sequence it may be said that *D. spinulosum* has a spinulose leaf throughout its life history, while *D. edule* shows the spinulose character only in the seedling, the later leaves gradually becoming entire. *D. Purpusii* has a leaf which is slightly but constantly spinulose in the adult plant. Its seedling is not known. Regarding the spinulose character of the seedling leaf of *D. edule* as due to recapitulation, *D. edule* is a later development than *D. spinulosum* and has come either from *D. spinulosum* or from some unknown form with spinulose leaves. The leaves of *D. edule* and *D. Purpusii* present about such characters as we should expect in *D. spinulosum*, if this species should be brought from its well shaded habitat into the dry hot places where the other two species are found.

I believe that throughout the Cycadales there has been a gradual reduction in the number of sporangia on a sporophyll. On this basis the sequence would be as I have given it.

On the other hand, it must be admitted that in *D. edule* the ovulate cone is not so compact and the megasporophylls have not lost so completely the character of the vegetative leaf, which must be assumed as the ancestral form of both kinds of sporophylls.

Since the original description of *D. spinulosum* was necessarily inadequate and incomplete, it seems worth while to describe the species from the more abundant material now available.

DIOON SPINULOSUM Dyer.—Adult plants 2–16^{m} in height; stem slender, with conspicuous transverse ribs, and a single zone of wood; leaves 1.5–2^{m} long, curved, with 80–117 leaflets on each side; leaflets 15–20^{cm} long, 10–15^{mm} wide, with 5–8 spines on each margin, the lower ones gradually reduced to mere spines; first leaf of seedling with as few as a dozen leaflets on each side, the leaflets with only 2–6 spines on each margin, lower leaflets not at all reduced; ovulate strobilus cylindrical ovoid, 35–50^{cm} in length, 20–27^{cm} in diameter; sporophylls densely hairy, closely imbricate, the exposed portion rounded or obtuse at apex; seeds smooth, white, 4–5.5^{cm} in length, 2.5–3.5^{cm} in diameter; staminate strobilus elongated ovoid, 21^{cm} in length, 10^{cm} in diameter; exposed portion of sporophyll hairy, obtuse; microsporangia about 750 on a sporophyll.

Observed growing wild at Tierra Blanca (State of Vera Cruz) and at Tuxtepec (State of Oaxaca), Mexico.

Material has been collected at intervals and a future account will deal with critical stages in the life history.

THE UNIVERSITY OF CHICAGO

THE INFLUENCE OF GRAVITY ON THE DIRECTION OF GROWTH OF AMANITA

STELLA G. STREETER

(WITH THIRTEEN FIGURES)

The following account is a record of a series of experiments carried on at the Biological Laboratory of the Brooklyn Institute at Cold Spring Harbor, under the direction of Professor D. S. JOHNSON, during the summers of 1906 and 1908. The object was to determine, if possible, the reactions of some of the common toadstools to the gravity stimulus. The main points under consideration are the promptness and accuracy of the response, the duration of the response after an efficient stimulus, the location of the zone of elongation, and its relation to the responsive zone. The conclusions here stated were drawn from observations made on about 3000 specimens, collected in the woods and replanted where conditions could be controlled. With the greatest care not more than one out of every ten planted yielded a satisfactory record. The agarics break easily, often from their own weight, when placed in a horizontal position; some shriveled instead of developing, and many were infested with the larvae of various insects, which fact was not apparent until the toadstool was near maturity.

The species used in these experiments were *Amanita phalloides* Fr. and *A. crenulata* Peck. These forms were chosen because they have long stipes, because transplanting seemed in no way to retard the normal development, and because they were very abundant. The food supply is stored in the button, so that the plant is not seriously affected by removal from the mycelium. The plants were collected from the woods just after they broke through the ground, at the stage when the pileus is beginning to break through the volva. Each was taken up carefully with some of its surrounding soil, carried to the laboratory, and there planted again in a tumbler. When it had been allowed to rest in the normal vertical position in a dark chamber for a short time, a careful drawing of each specimen was made in the following manner. The plant was placed, with the

stipe horizontal, at an accurately measured distance, before a sheet of paper firmly supported on a vertical drawing-board. In front of the toadstool and always at the same distance from it as the paper was fixed a black screen having a pinhole aperture. The drawing was made by looking through the opening and tracing the outline of the plant as it appeared against the paper. Other records were made in this way from time to time, depending upon the object of the experiment. The plant was in no way disturbed, and these records showed all changes of position in the vertical plane, and from them it was possible to measure these changes in units of angular measurements.

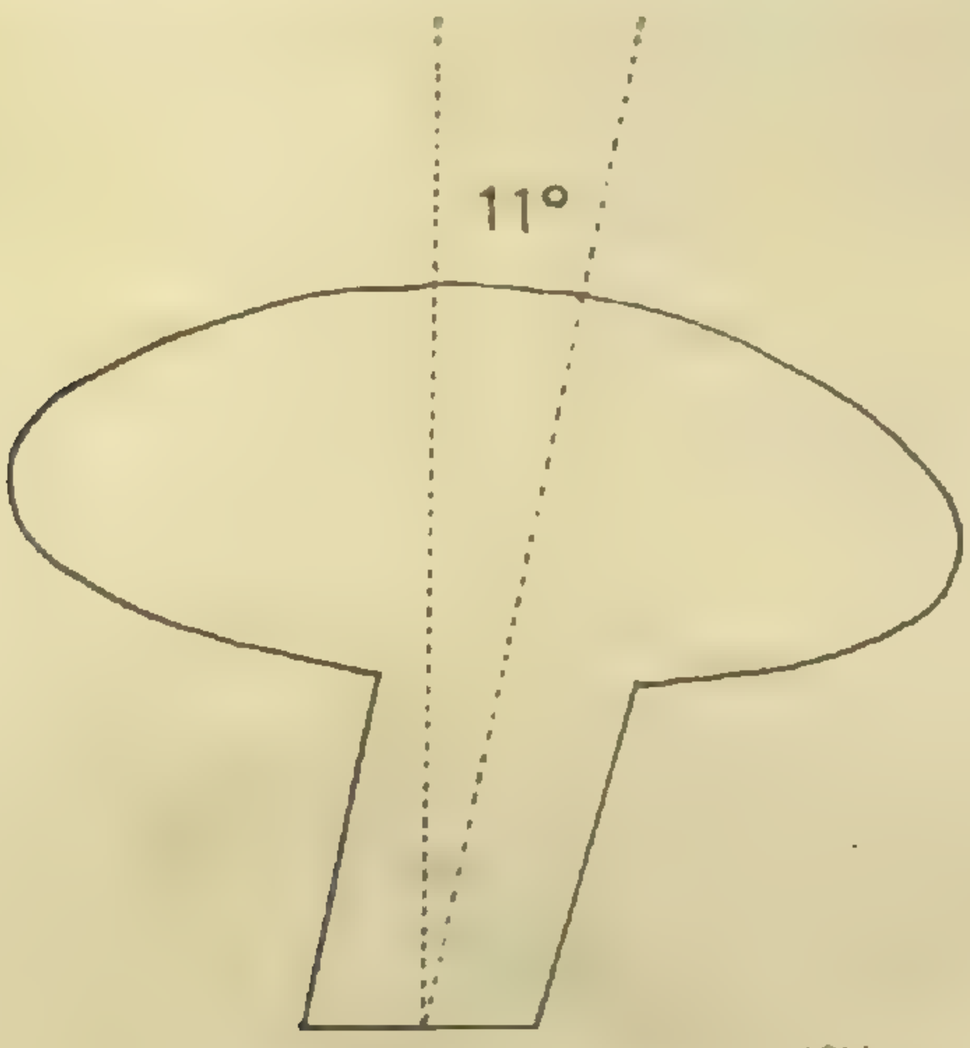


FIG. 1

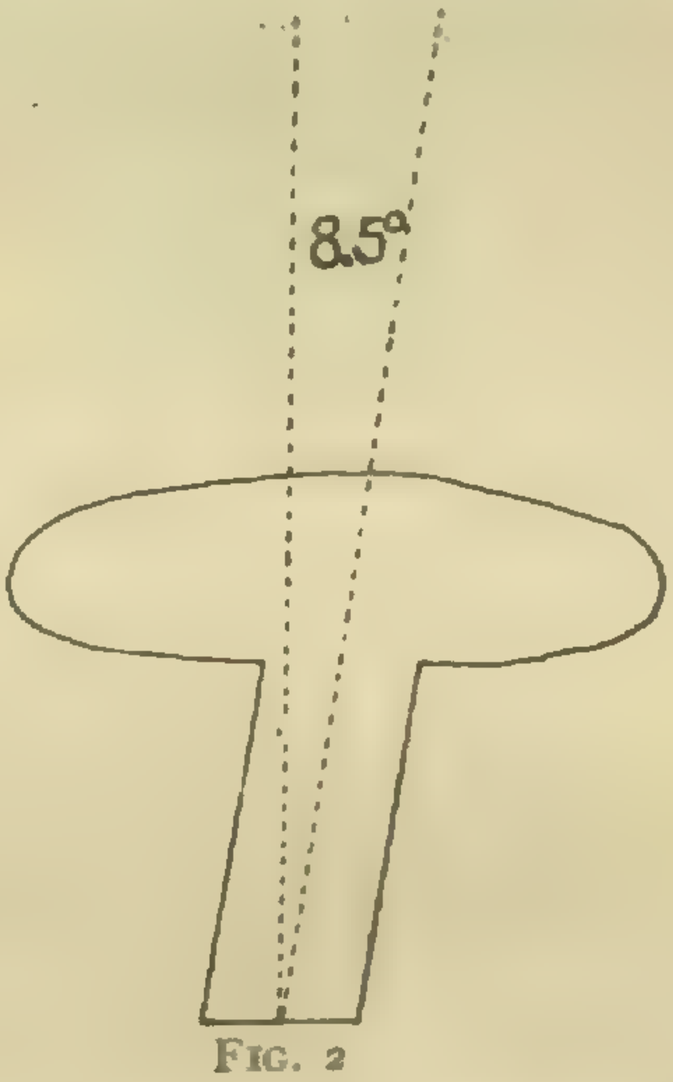


FIG. 2

FIG. 1. *A. crenulata*, 11° from perpendicular.—FIG. 2. *A. crenulata*, 8°.5 from perpendicular.

A few simple experiments showed that the forms used were positively heliotropic. Specimens planted in tumblers were placed in boxes which were painted black on the inside. One entire side of each box was left open and the boxes were placed in a west window with the exposed side toward the light. After standing in this position for 24 hours, the plants had bent 8° to 12° toward the light (*figs. 1, 2*). In *fig. 1* the main axis of the toadstool is shown inclined 11° from perpendicular, and in *fig. 2*, 8°.5. To avoid light stimulus, all experiments with reference to geotropism were performed within a moist dark chamber, which in turn was kept in a dark room.

After the pileus broke through the volva, it took the stipe about 24 hours to attain its full length in *A. crenulata*, and nearly 36 hours in *A. phalloides*. With a few exceptions this time element was con-

stant. This short period of development necessitated that all observations on one plant should be made within 36 hours.

In those experiments which required that the plant be held in a horizontal position, the tumbler containing the toadstool was supported firmly on a wooden block by a band of tin nailed securely to each side of the block (*figs. 3, 4*). A slender insect pin was placed in

FIG. 3.—*A. phalloides*, in which supra-curvature has been neutralized.

the center of each toadstool as a continuation of the axis of the stipe (also shown in the figures). This pointer made it possible to measure the amount of curvature accurately. After being collected and planted, each specimen was left in the normal position long enough to counteract any stimulation received during its transference and then it was placed on its side in the dark chamber.

Usually the pileus does not become fully extended until after the

stipe has ceased to elongate (*fig. 5*). In 24 hours after the toadstool had reached its maximum height, the pileus expanded and its diameter increased 1.2^{cm}. In all cases where the plant was placed on its side after the stipe had reached its full length, but before the pileus

FIG. 4.—*A. phalloides*, from which the pileus, except that part directly above the stipe, has been removed.

had fully opened, the pileus continued to expand; there was, however, no upward bend in the stipe (also shown in *fig. 5*). After the stipe attains its full length there is no longer any response to gravity stimulus.

When a young plant, in which the entire stipe is still elongating, is placed on its side in the dark, the tip of the stipe begins to bend

upward after 40–60 minutes. This curvature is continued for about 24 or 36 hours, depending upon the species, until the tip of the stipe

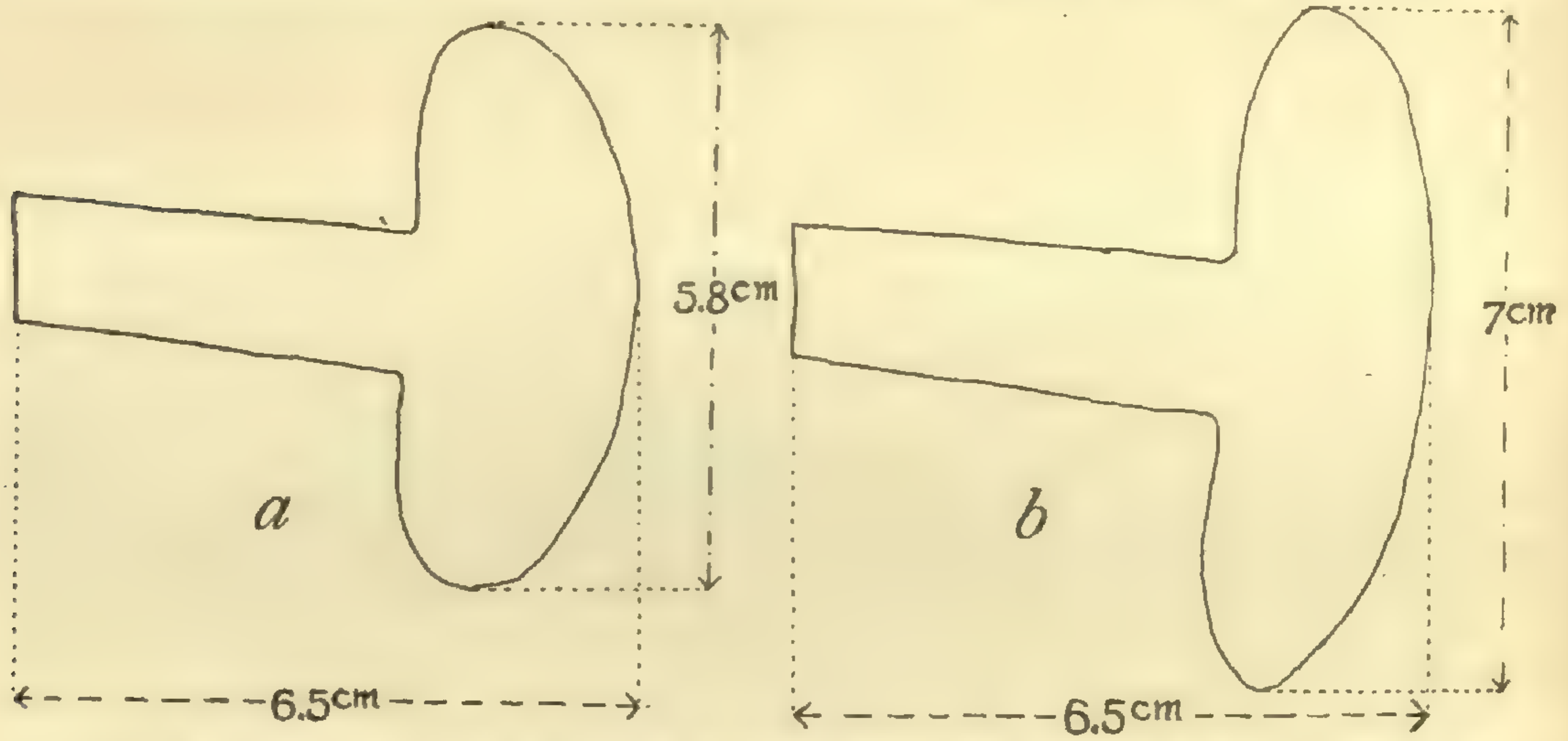


FIG. 5.—*A. crenulata: a*, height 6.5cm, diameter of pileus 5.8cm; *b*, 24 hours later, height 6.5cm, diameter of pileus 7cm.

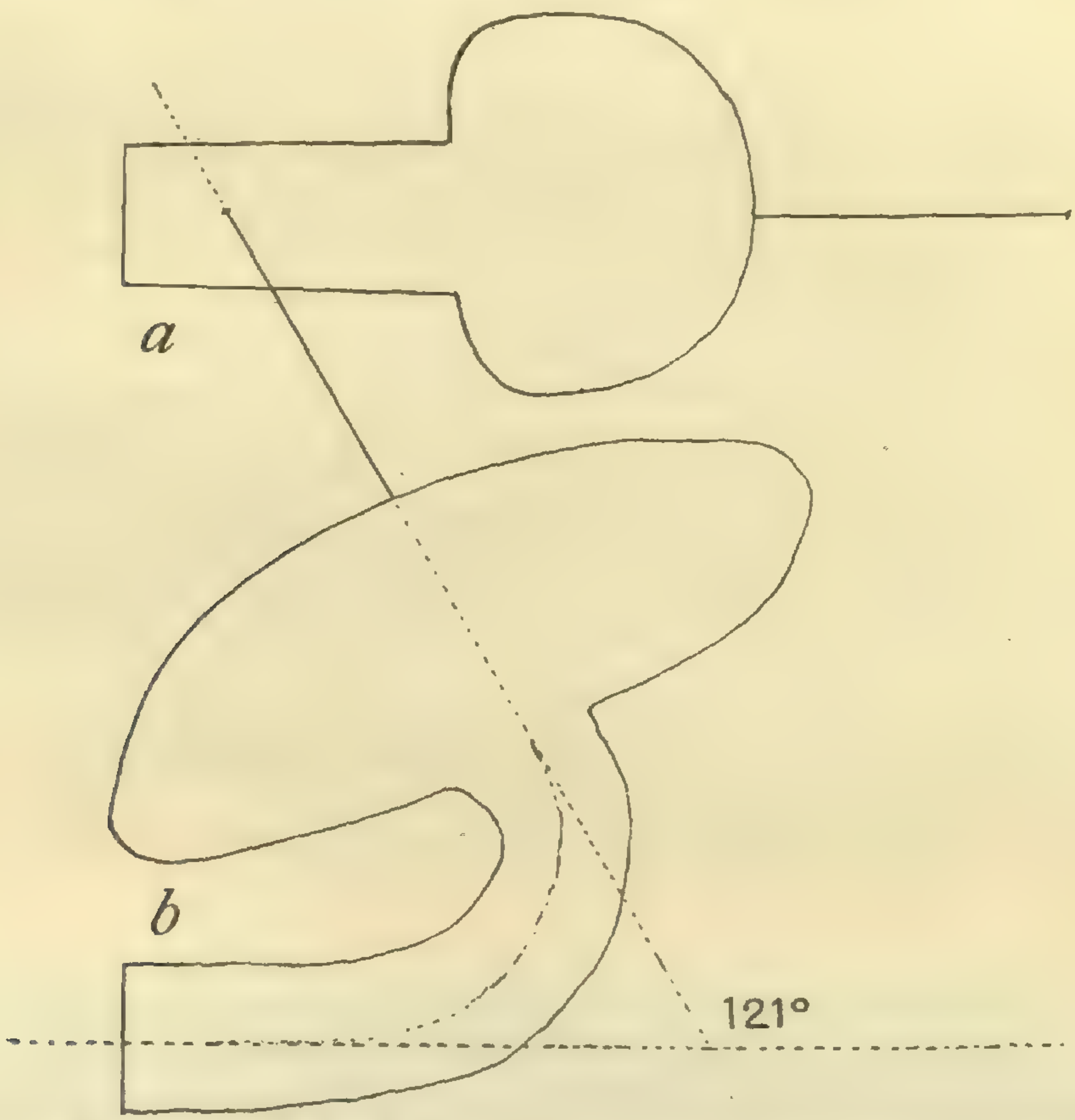


FIG. 6.—*A. crenulata: a*, as planted; *b*, 24 hours later, showing supra-curvature of 31°.

is carried up to and beyond the vertical position, and the original lower edge of the pileus is carried above the horizontal plane (*fig. 6*).

In experimenting with the stem of *Cephalaria procera*, one of the teasels, SACHS (1888) found that this supra-curvature was neutralized

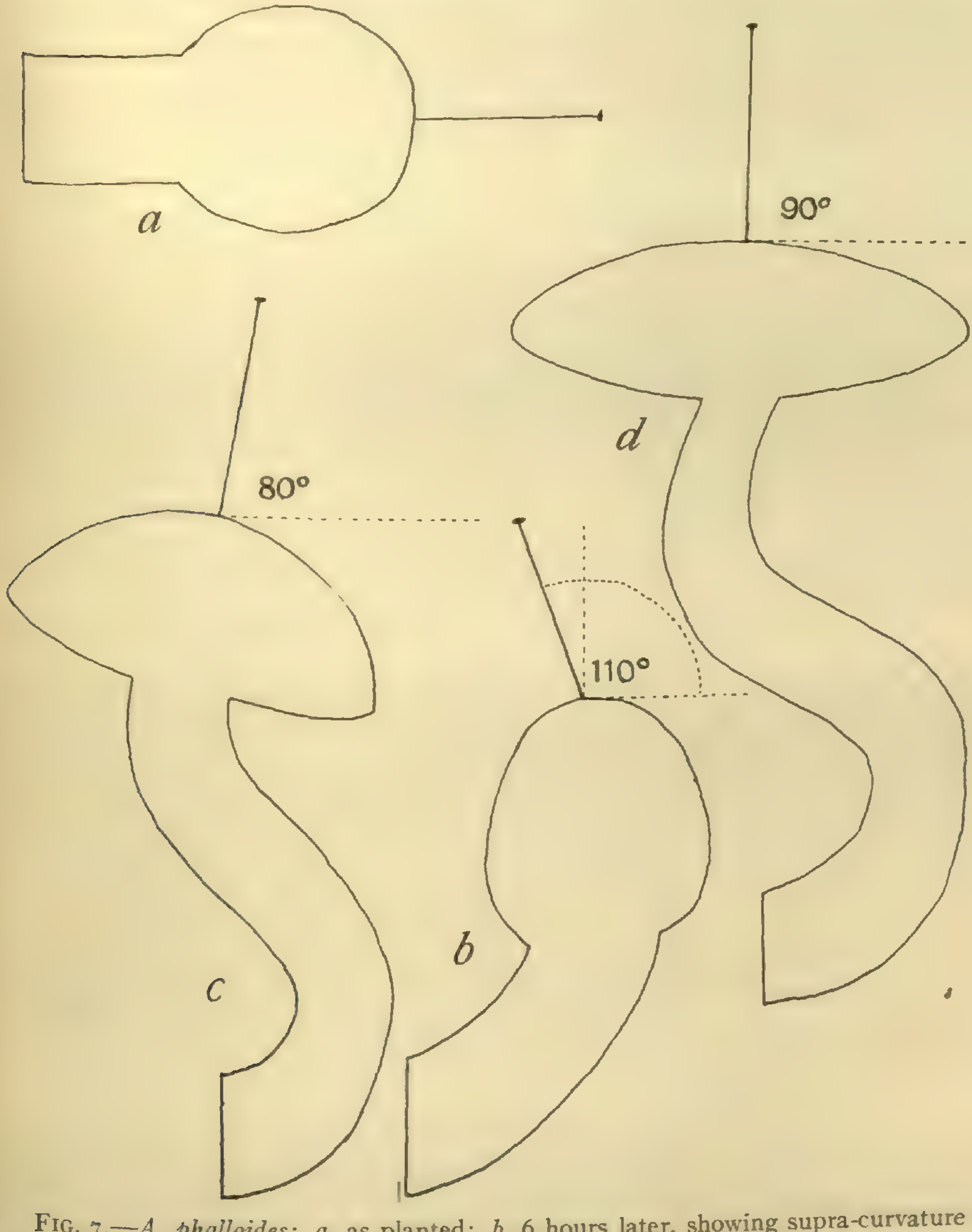


FIG. 7.—*A. phalloides: a*, as planted; *b*, 6 hours later, showing supra-curvature of 20°; *c*, 17 hours after planting, having returned to 10° below vertical; *d*, 30 hours after planting, tip of stipe vertical.

and the stem soon moved back to the vertical position. In the case of *A. crenulata* this supra-curvature is permanent. The probable explanation for this is that the period of elongation is so short that the reaction does not have time to take place before growth ceases.

When young specimens of *A. phalloides*, which have longer stipes and respond more quickly, were used, the tip of the stipe was carried beyond the vertical 2° to 20°, then it stopped and moved back toward and beyond the vertical 1° to 6° on the other side, and by a repetition of this process finally came to rest in the vertical position. *Fig. 7* shows a series of drawings of one specimen of *A. phalloides* in which the supra-curvature was neutralized. *Fig. 3* shows this toadstool just before it came to its final vertical position. This fluctuation only takes place if the plant is still growing when the induced response ceases.

In an attempt to locate exactly the perceptive zone, all the pileus except that part directly above and which forms a continuation of the stipe was removed in the manner shown in *fig. 8*. These plants were then allowed to develop in the dark in a horizontal position for 24 hours, and the result is shown in *fig. 8*. The stipe itself bent, but there was no curvature in that part which belonged to the pileus. This shows that the responsive zone is not situated within the pileus.

The entire pileus was then carefully removed from the tip of the stipe in many plants by a transverse cut where the gills join the stipe. Each specimen was then placed in a horizontal position in the dark and allowed to remain 24 hours. The geotropic response here was normal (*fig. 9*). The stipe, which was bent downward at first, began to bend upward slowly, then more rapidly, carrying the tip beyond the vertical. This shows that the responsive zone is situated within the stipe.

A further attempt to locate the responsive zone was made. Glass tubes were cut in pieces of different lengths and these were placed over the bases of the stipes of plants from which the pileus had been removed. This left 1–4^{mm} of the upper end of the stipe exposed beyond the end of the tube. These tubes were held firmly in a horizontal position by wire, and the plants were kept in the dark for 24 hours. In every case where elongation had not ceased, there was a decided upward curvature of the stipe beginning at the end of the glass tube (*fig. 10*). In this case the stipe was 8^{mm} long, 2^{mm} of this projecting beyond the end of the tube when the experiment was set up. After twenty-four hours the stipe projected 21^{mm} beyond the tube. It had curved upward at the end of the tube, making an

angle of 145° with the position from which it started. This shows that at least part of the responsive zone is very near the tip of the stipe. In other cases, where the stipe had nearly reached its greatest elongation, the result was the same (*fig. 11*). Here the stipe was

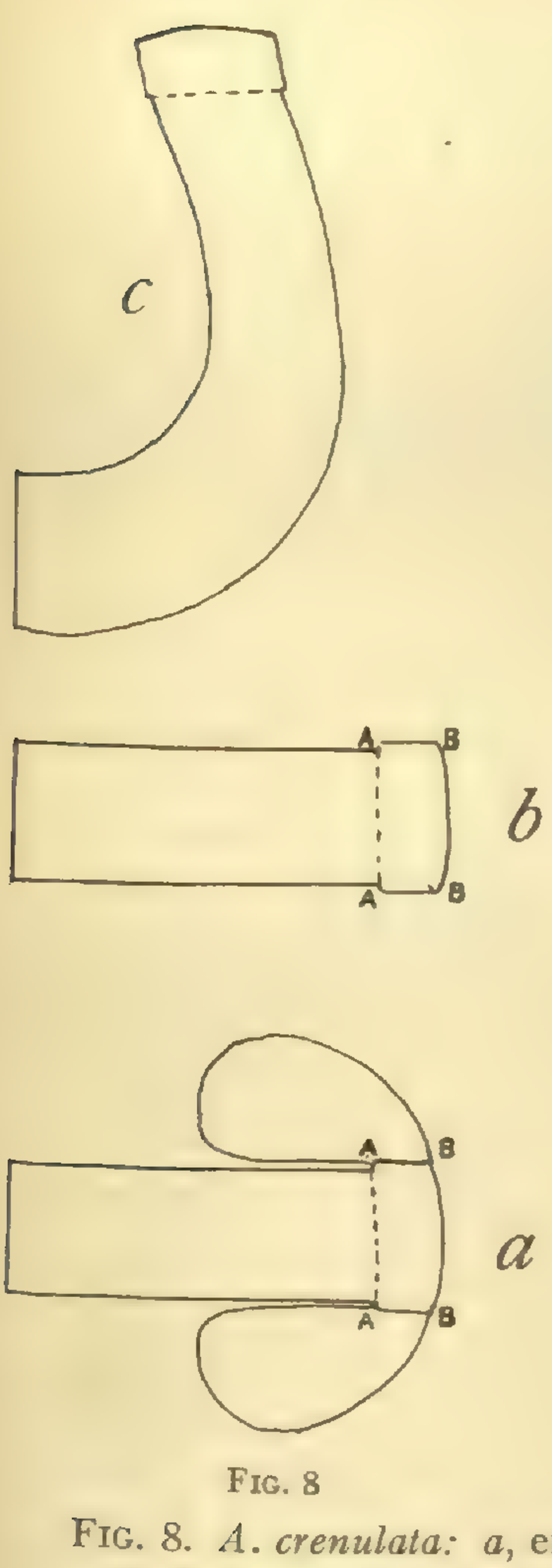


FIG. 8

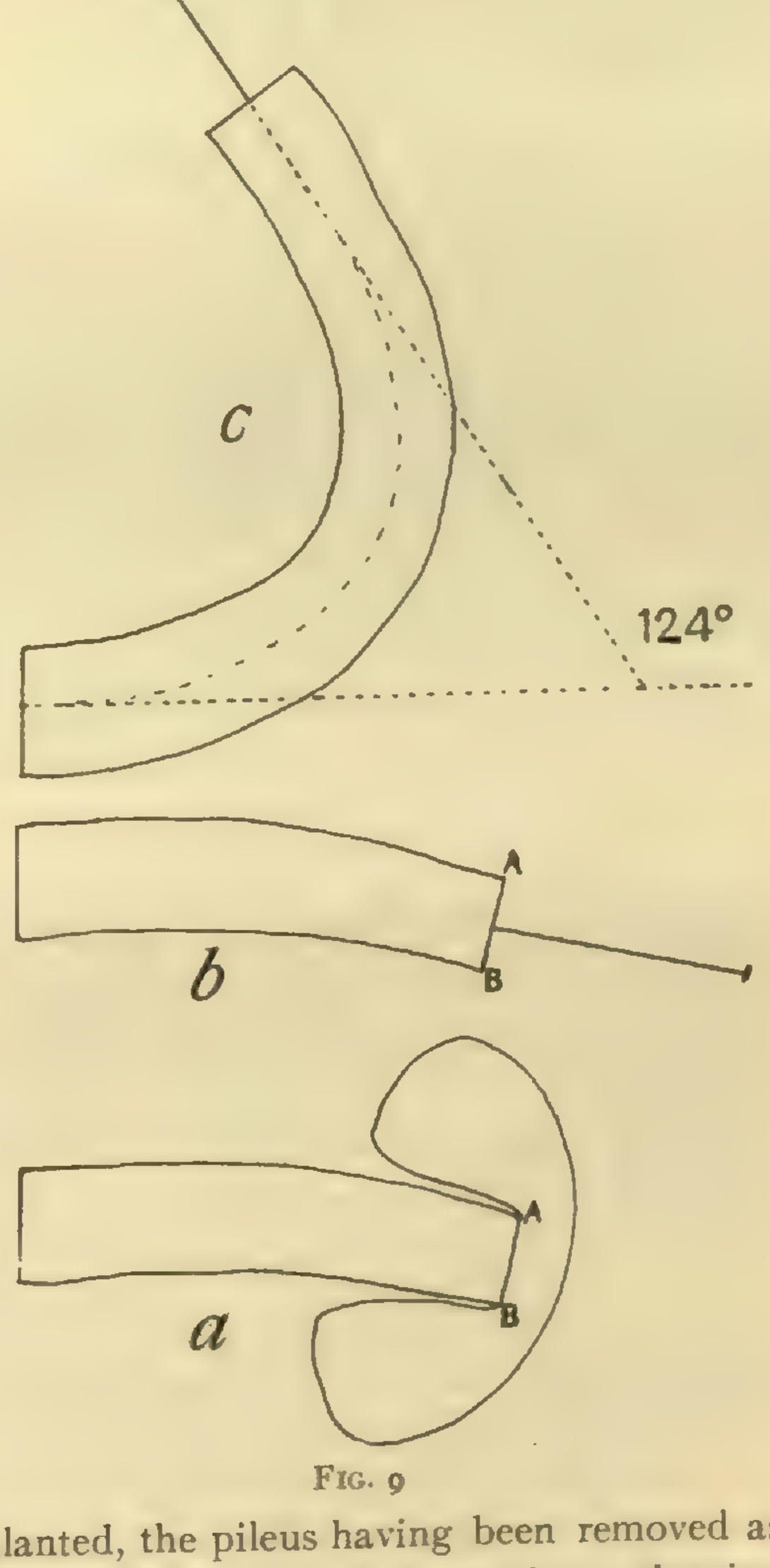


FIG. 9

FIG. 8. *A. crenulata:* *a*, entire; *b*, as planted, the pileus having been removed as indicated by the lines, dotted lines show depth of pileus; *c*, 24 hours later, showing that curvature takes place in stipe, not in pileus.—FIG. 9. *A. crenulata:* *a*, entire; *b*, as planted, the pileus having been removed by transverse cut *ab;* *c*, 24 hours later, showing curvature of 124°.

56^{mm} in length, of which 1^{mm} projected beyond the end of the tube. In this case elongation continued until the stipe projected 3^{mm}, and it then showed an upward curvature of 23°. In this case the responsive zone must have been within 1^{mm} of the tip at the start.

In order to determine the distribution of the zone of elongation,

the stipes of a great many specimens in all stages of development were measured. An accurate record of the length of the stipe at the beginning and end of growth was kept. The stipe was marked off into 2^{mm} lengths with dots of waterproof India ink. It was possible,

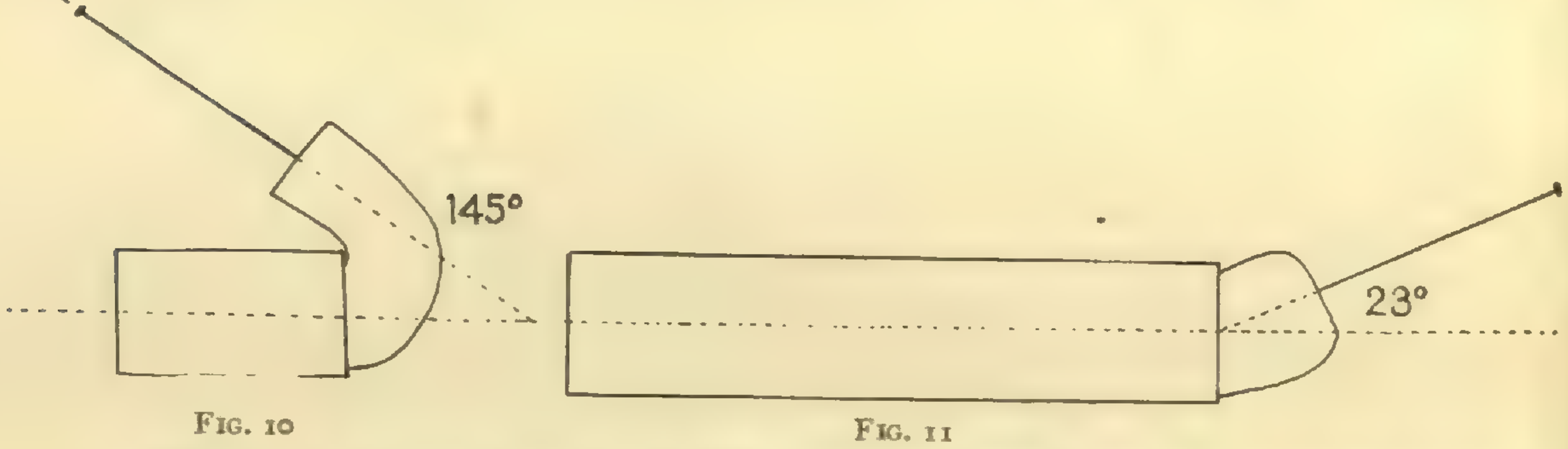


FIG. 10 FIG. 11

FIG. 10. *A. crenulata*, 24 hours after planting; base of stipe held in glass tube; tip curved 145°.—FIG. 11. *A. crenulata*, 24 hours after planting; base of stipe held in glass tube; tip curved 23°.

when growth ceased, to tell in what region elongation had taken place and where it was most rapid. A few of these records are given below.

Number	Length of stipe at the beginning	Length of stipe at the end	Amount of elongation	Elongating portion at tip	Non-elongating region at base	Elongation in each of uppermost 2^{mm} spaces beginning at top	In 2d five 2^{mm} spaces	In 3d five 2^{mm} spaces	In 4th five 2^{mm} spaces	Comparative length of stipe at beginning	Comparative length of zone of elongation	Time
	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	%	%	hours
110	7	33	26	7	0	7–10–5½–3½–0				21.2	100	36
216	20	50	30	20	0	3–3–3–3–5	4–3–2–2–2			40	100	36
218	30	68	38	30	0	2–2–3–3–4	4–4–3–3–2	2–2–2–1–1		44.1	100	36
157	40	72	32	40	0	3–4–3–3–2	2–2–1–1–1	1–1–1–1–1	1–1–1–1–1	55.5	100	36
104	20	34	14	20	0	1–2–2–2–2	1–1–1–1–1			58.8	100	36
142	30	42	12	20	10	2–2–2–1½–1½	1–½–½–½–½			71.4	66.6	36
148	42	52	10	20	22	1–2–1–1–1	1–1–1–½–½			80.7	47.6	36
138	102	104	2	8	94	½–½–½–½–0				98	7.8	36
123	36	36½	½	2	34	½–0–0–0–0				98.6	5.5	36

The specimens described above were grown in a horizontal position, and the measurements were taken on the lower side. Comparing the length of the stipe at the beginning and at the end of the experiment, and then comparing the length of the zone of elongation with the length of the entire stipe when the first measurement was taken, we find that elongation takes place throughout the entire length of the stipe until after it is 60 per cent. grown. From this time until

growth ceases, the zone of elongation becomes shorter and shorter, but is always just below the pileus. In the specimens used to make out the above table, the length of the mature stipe varied from 36 to 112^{mm}, and the length of the zone of elongation varied from 2 to 40^{mm}.

Record was made of the amount of elongation which had taken place in each part of the stipe beginning at the pileus. By referring to the table it can be seen that in those plants which are about one-third grown or less, the zone of most rapid elongation is in about the middle of the stipe. As growth continues, this zone moves up the stipe. In plants which are half grown or larger, the zone of most vigorous growth is the second 2^{mm} from the tip. When the zone of elongation becomes less than 4^{mm} in length, growth takes place in the upper 2^{mm} (see record for 123). The elongation within the upper 2^{mm} of the stipe is usually less than that within the second 2^{mm}, but in some cases they are equal. The amount of elongation in each 2^{mm} becomes less and less from the point of most rapid growth toward the base, until the growing region is passed.

In order to determine the time element in the geotropic response of these toadstools, plants were placed in a horizontal position in the dark and records of the amount of curvature were taken each hour. The following table gives the records of two of these specimens:

Time	No. 51		No. 56	
	Position	Amount of change for each successive hour	Position	Amount of change for each successive hour
7:30 A. M.	0°		0°	
8:30 A. M.	−4°	−4°	5°	5°
9:30 A. M.	1°	5°	15°	10°
10:30 A. M.	4°	3°	23°	8°
11:30 A. M.	8°	4°	32°	9°
12:30 P. M.	11°	3°	42°	10°
1:30 P. M.	15°	4°	52°	10°
2:30 P. M.	19°	4°	62°	10°
3:30 P. M.	22°	3°	72°	10°
4:30 P. M.	24°	2°	82°	10°
5:30 P. M.	26°	2°	88°	6°
6:30 P. M.	28°	2°	92°	4°
7:30 P. M.	30°	2°	95°	3°
8:30 P. M.	32°	2°	97°	2°
9:30 P. M.	32°	0°	98°	1°
7:30 A. M.	32°	0°	98°	0°

In about two-thirds of all the plants placed in a horizontal position in these experiments, there was a bending downward of 1° to 6° during the first hour, before there was any observable upward curvature. This may be partly due to wilting caused by transplanting. In general this downward tendency was less in the younger specimens where the stipe is shorter, and it was less where the pileus had been removed. These facts seem to indicate that it might be the physical effect of a new lateral strain rather than a response to a new stimulus. For this reason in many cases the pileus was removed, as is shown in *fig. 4*. No. 51 shows this bend downward in the first hour, followed by 1° more than complete recovery in the second hour. For the next six hours the rate of curvature fluctuated from 3° to 4° per hour; this was followed by a steady rate of 2° per hour for five hours; then the pileus came to rest at an angle of 32° from horizontal. In No. 56 the response during the first hour was slight; during the second it was greatly accelerated; this was followed by a period of depression which lasted for one or two hours; from this time on for the next five or six hours the reaction was constant and rapid, then more slow. After the vertical line was passed, the curvature took place more and more slowly until, in the case cited here, it came to rest at an angle of 98° from its original position. Nine hours later, or 24 hours from the start, it remained in the same position and there had been no increase in length. There was no neutralization of the 8° of supra-curvature.

In order to determine the exposure period, that is, the length of time which the plant must be stimulated in order that reaction may follow, several plants were kept in a fixed horizontal position for 15 minutes and were then rotated on a clinostat about the horizontal axis for 24 hours. The plants gave a decided reaction to geotropic stimulation for this length of time. There was an upward curvature of the stipe varying from 15° to 30° from its position during exposure to the stimulus. Four records of plants of *A. phalloides*, stimulated by being in a horizontal position for 5 minutes and rotated on a clinostat for 24 hours, showed an upward curvature of 7°, 10°, 10°, and 15°, respectively. Eight records of plants of both *A. phalloides* and *A. crenulata* stimulated for one minute all show an upward curvature of either 6° or 7°. In one case of stimulation for 5 minutes,

in three cases of stimulation for one minute, and in all cases (ten records) where the specimens were in a horizontal position only 30 or 15 seconds, there was no direct upward curvature. Instead, the stipe had made a definite spiral curvature in the same direction as that in which the clinostat moved. Clinostats which rotated in opposite directions were used and the spiral always followed the direction of the clinostat (*fig. 12*).

To find the latent period, the pileus was carefully removed that curvature might not be retarded by the weight which would cause a strain in an unusual place and the plants were placed in a horizontal position. Records were made every 10 minutes. In young, vigorously growing specimens, there was an upward curvature observable in 40 minutes. In other specimens which were nearer maturity, the period was longer, in some cases being 60 minutes.

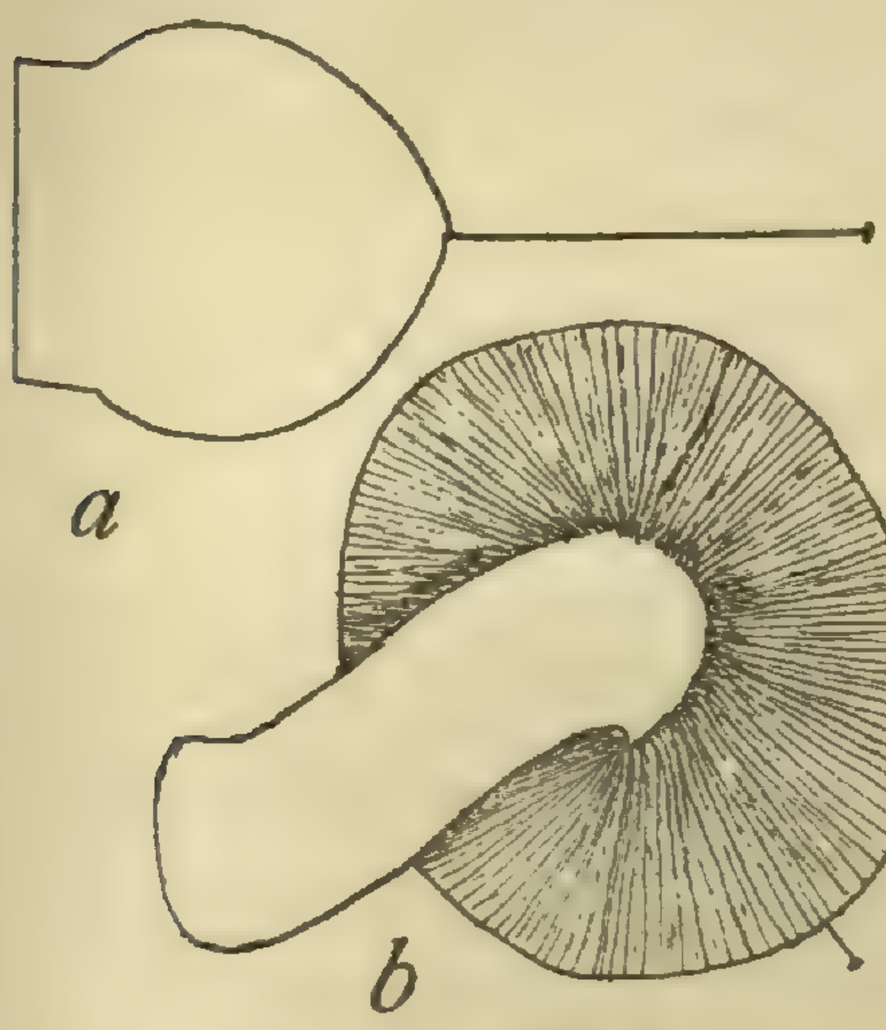


FIG. 12

FIG. 13

FIG. 12. *A. phalloides: a*, as placed on clinostat; *b*, 24 hours later, showing special curvature of stipe.—FIG. 13. *A. phalloides*, showing amount of curvature for each 10 minutes for 80 minutes.

No records which showed the downward bend were considered in this experiment. The diagram (*fig. 13*) shows the rate of curvature in a typical specimen.

SUMMARY

When young and vigorously growing toadstools were placed with the stipe in the horizontal position, the stipe of each toadstool bent and carried the pileus up to or beyond the horizontal position. This supra-curvature when it occurred was neutralized if growth did not cease too soon.

The responsive zone is situated near the tip of the stipe, not within the pileus.

The stipe elongates throughout its entire length, until it is more

than half grown. The zone of most rapid elongation is always just below the pileus and becomes shorter and shorter until growth ceases.

When placed in the horizontal position, the tip of the stipe curved upward very slowly at first, then more rapidly, until it passed the vertical position, after which the curvature took place more slowly until it came to rest. If growth were still vigorous, the tip of the stipe again passed the vertical, rested on the other side, and finally assumed the ordinary position.

The amount of time which a toadstool must be stimulated in order that reaction may follow is less than a minute. How much less was not satisfactorily determined, probably because the clinostats used rotated too slowly.

The latent period varied from 40 to 60 minutes, the younger specimens responding more quickly.

JERSEY CITY, N. J.

THE WILLIAMSONIAS OF THE MIXTECA ALTA

G. R. WIELAND

(WITH TEN FIGURES)

The great plateau and mountain region in the central and western portion of the southern Mexican state of Oaxaca is commonly known in Mexico as the Mixteca alta, or high country of the Mixteca Indians. This name is used in contradistinction to the Mixteca baja, or portions of the lower country or tierra caliente over which the Mixtecas have extended. Simply speaking, then, the Mixteca alta is a portion of the southern edge of the Cordilleran system facing the Pacific and extending through western Oaxaca to the border of Guerrero, or into the latter state. Here, during some five months of the past winter and spring I have had the pleasure of continuing field work on the American fossil cycads under the auspices of the Instituto Geologico Nacional de Mexico. I have the kind permission of the director, Señor AGUILÉRA, to note in this preliminary manner that the results obtained are regarded as of the greatest interest to paleobotanists.

The field work has included the making of an accurate section through fully 2000 feet of plant beds of Rhät-Liassic age, full of cycads in as great a variety of types as has thus far been encountered anywhere in the world. Indeed, considering the fine material obtained from the localities noted in the valley of the Rio Nochixtlan near Tlaxiaco, at Mixtepec on the Rio Tlaxiaco, notably all around the Mina Consuelo, about El Cerro del Lucero, and the Rosario region to the southwest of Tezoatlan, as well as elsewhere, and considering, moreover, the almost endless opportunity for opening up quarries in all this extensive country, I am of the opinion that the Mixteca alta is one of the most promising and accessible regions for the student of fossil plants yet discovered.

True enough, these localities are distant from the railway 50 to 150 miles or more, over the roughest of mountain trails. And as mountain succeeds mountain with scarce a valley between, there are some real difficulties for the collector, who must send out his material in blocks of limited size, such as can be loaded on the backs of the

tough little burros, or on the strongest mules, which can carry a pair of boxes of 200 pounds weight each. Hence, it more than once becomes necessary to break the splendid slabs all covered with leaf impressions, and often bearing flowers, which one can secure in even the smaller quarries almost without limit.

But for a country so distant, there are many very distinct advantages and no final obstacles. Animals are trusty; the Indians are good and cheap workmen; one is everywhere met with great courtesy; it is a source of much interest to find the high degree of comfort to be had in some of the most distant of the towns and villages; the food is always good, and even in the far mountains one can always get eggs and tortillas. All in all, if one is only fairly fore-wise, to spend a winter in the sunshine, the forests, and amidst the grand scenery of the Mixteca alta is much more like enjoying oneself in some geological sanatorium, as it were, than like hard work. For the naturalist ever finds a thousand and one points of interest, and soon becomes accustomed to ride 20 to 50 miles a day as he may need; while if interested in the cycads he can find places farther down the cañons and deep valleys where species of Dioon are fruiting hard by strata yielding the fossil forms in profusion.

The great thickness of these Oaxacan plant beds has been noted; but as to their exact age I am not sure, it being as yet early to form a fair conclusion. Glossopteris, rather than merely the sagenopterids, is considered present; and there is also a wealth of taeniopterids of older type, as well as a fine series of stems of a small but distinct lepidodendrid, and many leaves of *Noeggerathiopsis Hislopi* Bunbury. But otherwise the facies is uppermost Triassic, if indeed Liassic genera may not in the end be found to preponderate. A similarity to the Gondwanas of India suggests itself.

But what commands our attention at present, far beyond the precise age of these beds, is the fact that there abound in them in great variety the imprints, casts, and molds of many fruits of Williamsonia, closely associated with Zamites, Otozamites, Podozamites, Pterozamites, Ptilophyllum, and Dictyozamites, fronds as well as seeds. One of the strobili is a mold of just such an ovulate fruit as BUCKLAND figured under the name of Podocarya in the *Bridgewater treatises*, the original of which should be at Oxford, but could not be

found when a search was made for it a few years since, as Professor SEWARD of Cambridge has informed me. Also, there is a considerable number of the large buds inclosed by heavy ramentum-covered bracts of the same size and appearance as those from the Yorkshire coast. One favorable circumstance that has conduced to frequent preservation of the surface characters of the ovulate fruits is the formation by the outer zone of a layer of coal, which while liable to checking affords an excellent indicator for which one may keep constant watch. Furthermore, the close association of these fruits, leaves, and stems, though the latter are not well preserved as a rule, leads to the hope that as the collections come to embrace a wider range of localities, and the data of association come to be better known, more than one restoration of the complete plant can be made. That silicified forms may yet be found is proven by the occurrence of a well-silicified log of a new species of Araucarioxylon.

Especially interesting is the occurrence at Mixtepec on the Rio Tlaxiaco of fruits of small size borne on slender stems, and also those with broad bladelike bracts of thin texture, if they are not indeed sepals or petals. These small fruits, while not preserved in finer details, are abundant, and are quite uniformly accompanied by small, much-branched stems, and by numerous fronds no more than 10^{cm} in length; though these may have been bipinnate.

Of primary importance is a single fairly well-preserved impression of a staminate disk from midway up in the plant beds in the main barranca between El Cerro del Venado on the south and El Cerro del Lucero on the north, near the coal outcrops of Mina Consuelo, 15 miles from Tezoatlan. On my return from the Mixteca alta I supposed that I had not found any of the staminate organs of the cycadophytes; although various interesting fruits of seed ferns appeared to be present. But I found on my desk at the Instituto Geologico a letter from Professor NATHORST, dated from England and telling me that he had just visited the Yorkshire coast, where he had succeeded in finding the first definitely recognizable male flowers of Williamsonia, these agreeing essentially with the flower of Cycadeoidea as first discovered in the type of *C. ingens* and described in my *Studies of American fossil cycads*, parts I–IV.

It can be readily understood that I had kept a sharp watch for

such disks during all the field work, and that after reading Professor NATHORST's letter this watch was made closer still while the various specimens were being unpacked, placed in order, and further developed. But not until all this work had been done did I finally, in an idle moment, uncover a staminate disk on a slab from near Mina Consuelo. Then I recognized that a form I had suspected at one time was a disk had been truly such, though poorly preserved. It seems I had not chanced on quite the best disk locality. Better localities will yet be found.

The El Consuelo Williamsonia staminate disk is a reduced campanulate form of the size and general structure indicated in *fig. 2*. As there shown, however, the number of fronds is arbitrarily taken for purposes of interpretation as five, instead of the true number of eight or ten. One cannot be quite sure of the exact number in the specimen. The important structural feature, however, is that instead of a bipinnate frond as in Cycadeoidea,[1] there is a small strictly once-pinnate form, the rachises bearing only two lateral rows of synangia. These, however, are still of the marattiaceous or cycadeoidean size and structure. The component fronds project beyond the disk only to a height of about 1.5^{cm}. The basal region appears to be rather thin in texture; but the state of preservation as well as the association with other fruits and leaves all go to indicate that these disks and doubtless those of other species and genera should yet be found in abundance.

There can be no gainsaying the supreme importance of this fossil flower in enabling us to form more exact and adequate conceptions of the course of evolution leading up to and resulting in the present diversity of gamopetalous plants. In describing the flowers of *Cycadeoidea ingens* and *C. dacotensis*, I pointed out that the disk type of cycadophytean flower clearly indicated previous stages with the staminate organs spirally inserted beneath an apical cone or series of likewise spirally inserted megasporophylls, and that readily conceivable reductions and changes in such a primitively organized inflorescence or fertile apex or branch fully indicated the mode of origin of Liriodendron. Whence it followed that the Magnoliaceae

[1] I followed the old usage of calling these fronds pinnate in my descriptions; but ARBER has called them bipinnate forms. It seems rather more correct so to do.

must be among if not the most primitive of all the angiosperms. In this I believe I was clearly the first; though HALLIER soon independently reached similar conclusions as to the origin of the Magnoliaceae, and likewise cited Liriodendron.

Later, ARBER and PARKIN visualized in the *Annals of botany* the conception of a primitive semi-cycadean angiosperm ancestor with its crown of reduced, once-pinnate, spirally inserted microsporophylls surmounted by similarly set carpophylls; and these authors at the same time carried much further the idea that the true mode of angiosperm evolution, so long completely hidden, was at last indicated in all its broader outlines by reasonably direct evidence.

In ARBER and PARKIN'S ancestral stage we certainly behold a very primitive and decidedly plastic type, which they choose to call a hemiangiosperm, though I perversely prefer to group such plants under SAPORTA'S old term of proangiosperm.[2] It is one of the hypothetical forms, moreover, which is not only so readily conceivable as undergoing an infinity of modifications in the direction of higher forms, but one which, as I have long since told ARBER, we may confidently expect will yet be found in the fossil stage.

Despite all this progress, the actual mode of modification leading into the more complex types of angiospermous flowers has remained

[2] From a long letter that ARBER has been kind enough to write me, I know his views on the question of whether in hypothesizing the more immediate angiosperm ancestors we may use the old term proangiosperms, or must define a new and different group, the "hemiangiosperms," as he has proposed. I duly respect these views and think it is of importance that they receive a more thorough consideration than can be accorded them here.

At the same time, I wish to point out that we should duly honor SAPORTA as one of the pioneers, who, whether clearly or not, glimpsed an important truth when he placed Williamsonia among his proangiosperms. To claim that the proangiosperms of SAPORTA, as a great and more or less hypothetical group, either had to be, or in his day could have been delimited would to my mind be both impractical and unjust. Moreover, the important point is that we may well allow that SAPORTA was taking a glance backward in the direction of primitive forms even more than forward. Indeed, it is both fair and convenient to remember that the proangiosperms, as a great group leading into the angiosperms, could have their gymnosperm section, and yet logically include other truly lineal families, these being of hardly more than family distance from the Cycadeoideae at best. For these reasons, partly pertinent at least it seems to me that to use fairly the term hemiangiosperm we must go much farther back, to where by another hiatus the proangiosperms merge into the more primitive fern derivatives.

obscure. While SCOTT, especially, as well as HALLIER, ARBER and PARKIN, BESSEY, and others, as well as myself, have all insistently urged that the path has thus really been blazed to a knowledge of the evolution of the angiosperm groups, no one has yet been in a position to bring the final methods more clearly into view. Now, however, I believe we can do so in the case of all but the extremely modified families, if not indeed ultimately in these too, by means of analogic methods combined with comparative morphology.

We have seen how the staminate disk of *Cycadeoidea ingens*, and of all the other species so far known, bears inside the campanula or androecium many functional synangia on the rachial axes, either inserted directly or on pinnules. Now we see, furthermore, that the campanula remains large in a form with greatly reduced fronds, while we may be sure that interiorly borne synangia are still present on the rachial axes, or at least may be in related forms, whether we can detect them or not. Whence it follows that a slight further continuance of the rachial reduction, so plainly under way, must result in a toothed campanula bearing interiorly on each axial or rachial line only a single pair of synangia and finally a single synangium and no more, just as diagrammatically shown in *fig. 3*. Such types we may call, because of the strictly convolvulaceous aspect, *archaeosolandrous*, arbitrarily dismissing for the present all mention of the central ovulate region to which we return below.

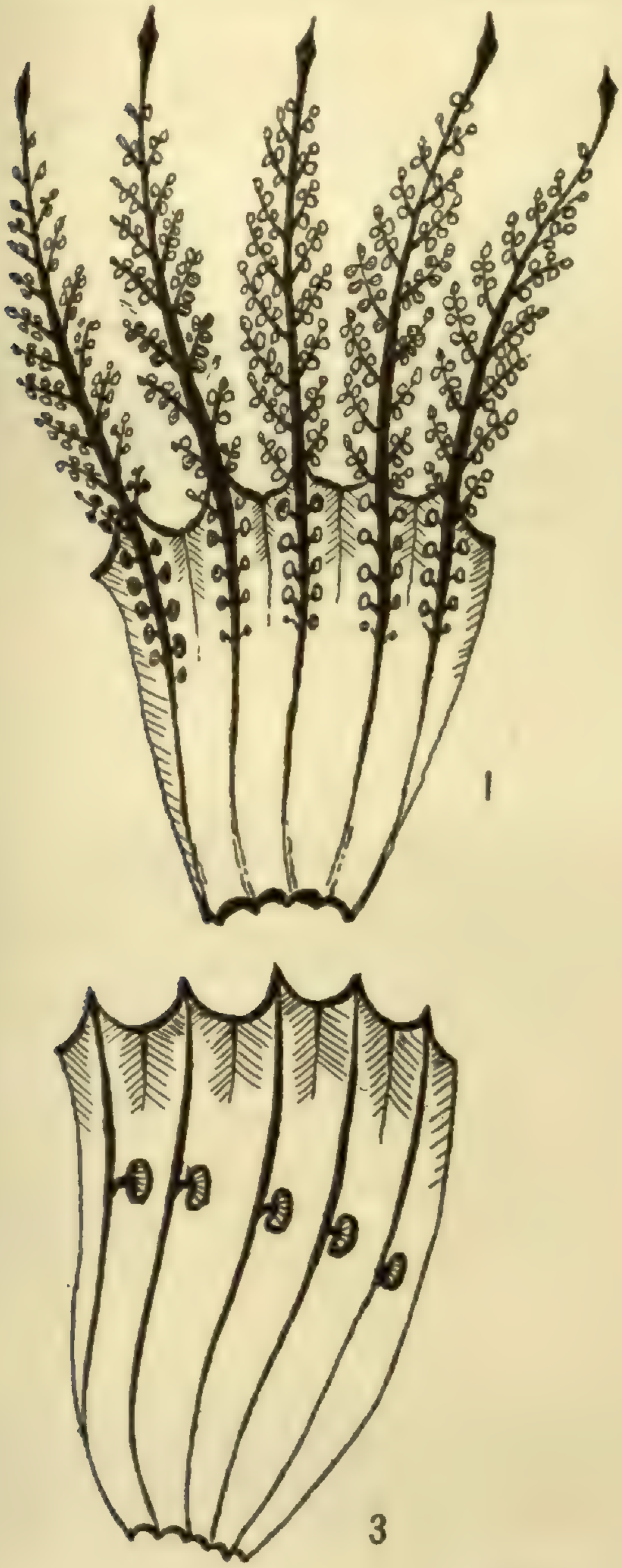
Now plainly, having thus by a series of entirely simple stages reached this archaeosolandrous form, which we confidently believe will be detected in the fossil condition, it is only a minor step to simpler types of pollen sacs, either borne in pairs or singly, and finally to elongations of the filaments suiting the requirements of such flowers as that of *fig. 4*, which is none other than a common morning glory.

In short, the successive members of the series of steps outlined are these, letting capitals represent the known, and small letters the hypothetical plants:

A′. Proangiosperms, or hemiangiosperms and pteridosperms.
A. The flower of *Cycadeoidea dacotensis*, with a campanulate disk of 18 bipinnate members.
B. The flower of *C. ingens*, with 12 bipinnate members.

C. The flower of *C. Jenneyana*, with only 10 bipinnate members, as well as various other greatly reduced and small types of flowers of Cycadeoidea.

d. A hypothetical flower of Cycadeoidea, with a staminate disk of 5 bipinnate members or staminate fronds (cf. *fig. 1*).



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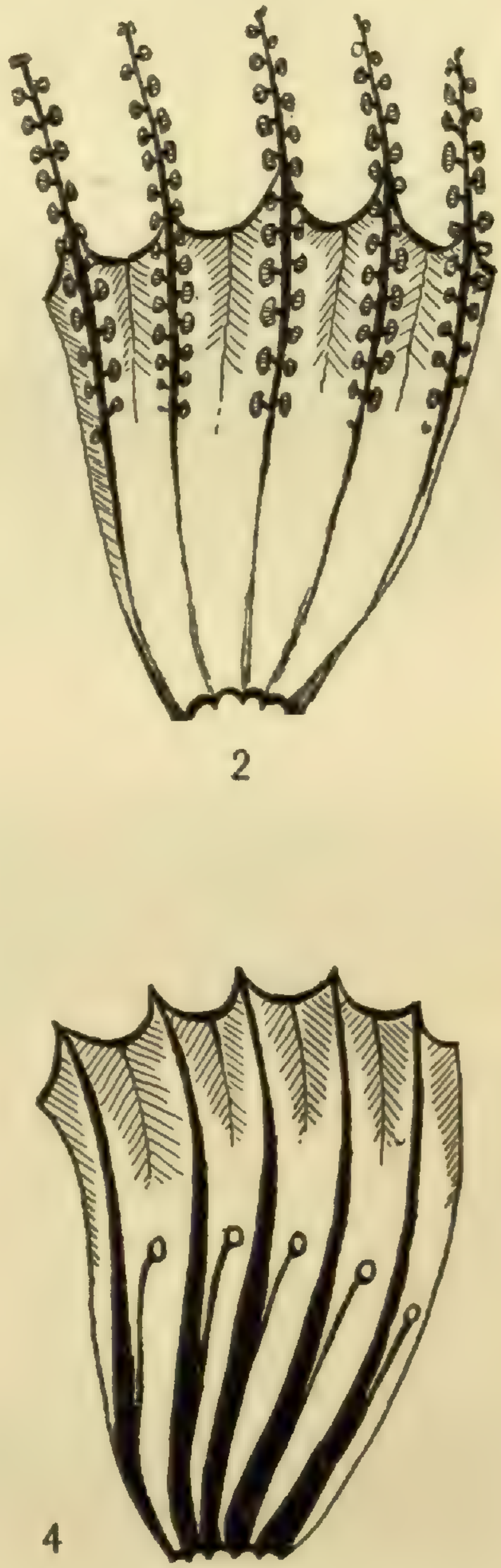


2

3

4

FIGS. 1–4

E. The El Consuelo staminate disk composed of 10 or 12 pinnate fronds.

f. A derivative of the El Consuelo disk, with only five pinnate fronds forming the campanula (cf. *fig. 2*).

g. A hypothetical campanula derived from the preceding by reduction of the synangial number to two and finally one, borne interiorly (*fig. 3*).

h. Unknown intervening plants derived from *g* and undergoing reduction in the number of the sporangial loculi, with thinning of the synangial wall, or

i. An unknown form much like *g*, but with a simpler type of pollinial organization. (This is the more probable member of the direct series, but both may be conceived of as having once existed.)

j. A campanula derived from either *h* or *i*, in which reduction to the angiospermous pollen sac has taken place, and in which elongation of the staminal pedicels or filaments is going on (cf. *fig. 4*).

K. The staminate campanula of the convolvulaceous and many other gamopetalous flowers.

Thus may we derive the staminate zone of gamopetalous forms by a series of readily conceivable and closely united reduction stages, the principal members of which are in the larger sense already known. But let us now see if it be possible to go on and establish a plausible ovulate correlation; for if this cannot be done it is more than superfluous to say that *K* is in the extended sense not proven to be a true member of the evolutionary series *A′*, *A–j*.

That an apical series of spirally inserted carpophylls giving rise to a central ovulate cone played the chief rôle in the development of the Magnoliaceae, as in all the conifers and doubtless many other forms, is evident enough. But that such a cone was in *all* cases organized, or much less that a terminal group of diffuse carpophylls was present in all the ancestral phyla of the angiosperms, is after a little consideration seen to be a cumbrous hypothesis. By what other means then than by the reduction of the carpophylls and of cones may we conceive of the cognate origin of the ovulate region in angiosperms?

Perhaps the Draceneae may be made to give an initial answer. Take for instance the cultivated maguey (Agave) of Mexico, with its six immense versatile anthers borne on their long projecting filaments as seated in the interior surfaces of the six fused sporophylls, three of which are smaller, and the other three of which are alternately of larger size. On cutting this flower open and viewing it from the interior, is not the structure in reality that very diagrammatically indicated in *fig. 5?*

Now, was not this floral structure derived from a consolidating and changing whorl of six primitive bisporangiate fronds with basal megaspores and apical microspores? The simple method of deriva-

tion from such a primitive, or *archaeo-amarillidaceous* plant would consist in basi-lateral fusion of the sporophylls and development of a trilocular ovary, with the synchronous reduction and change of the microspore zone to the series of six interiorly borne stamens, after the manner shown to be feasible in the above series *A′*, *A–j*. Such we conceive to have been the origin of the yuccas and agaves; for it is no more improbable that heterospory should in many primitive stocks arise thus regularly in each of an apical whorl of sporophylls, than that it should find expression in the segregation of a basal group of megasporophylls followed by an apical group of microsporophylls, as in Cycadeoidea.

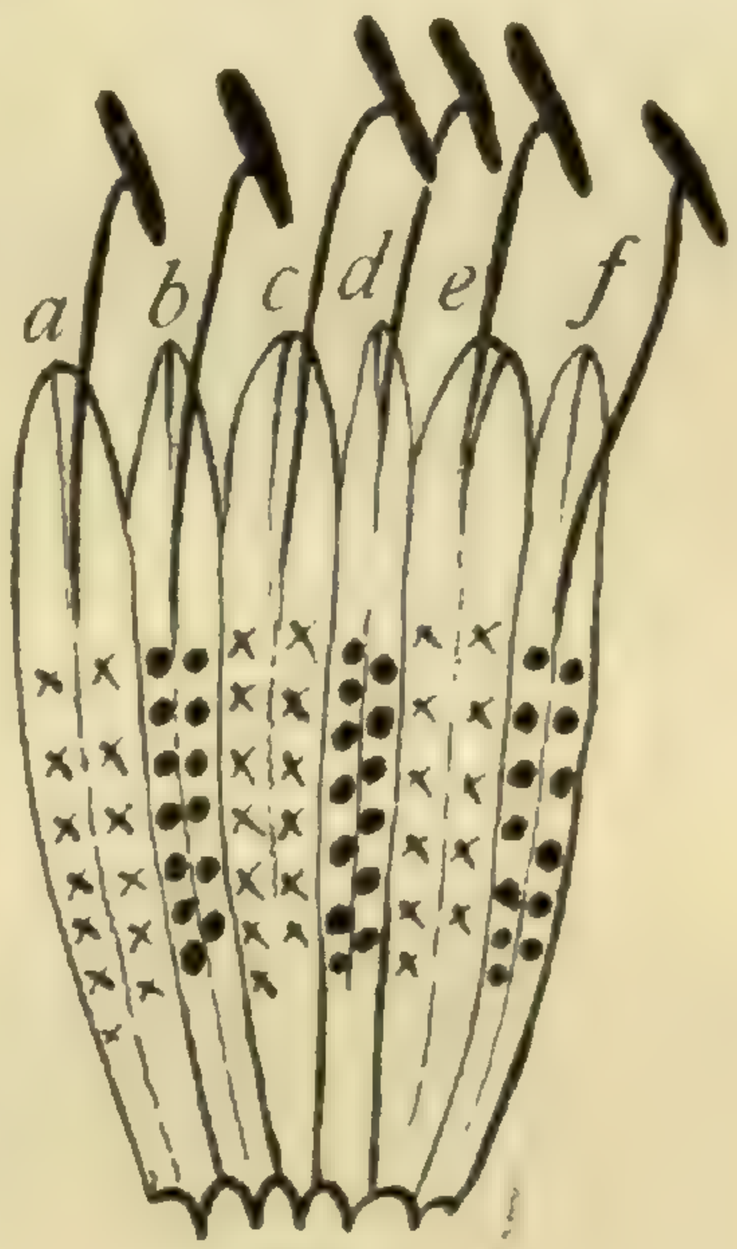


FIG. 5

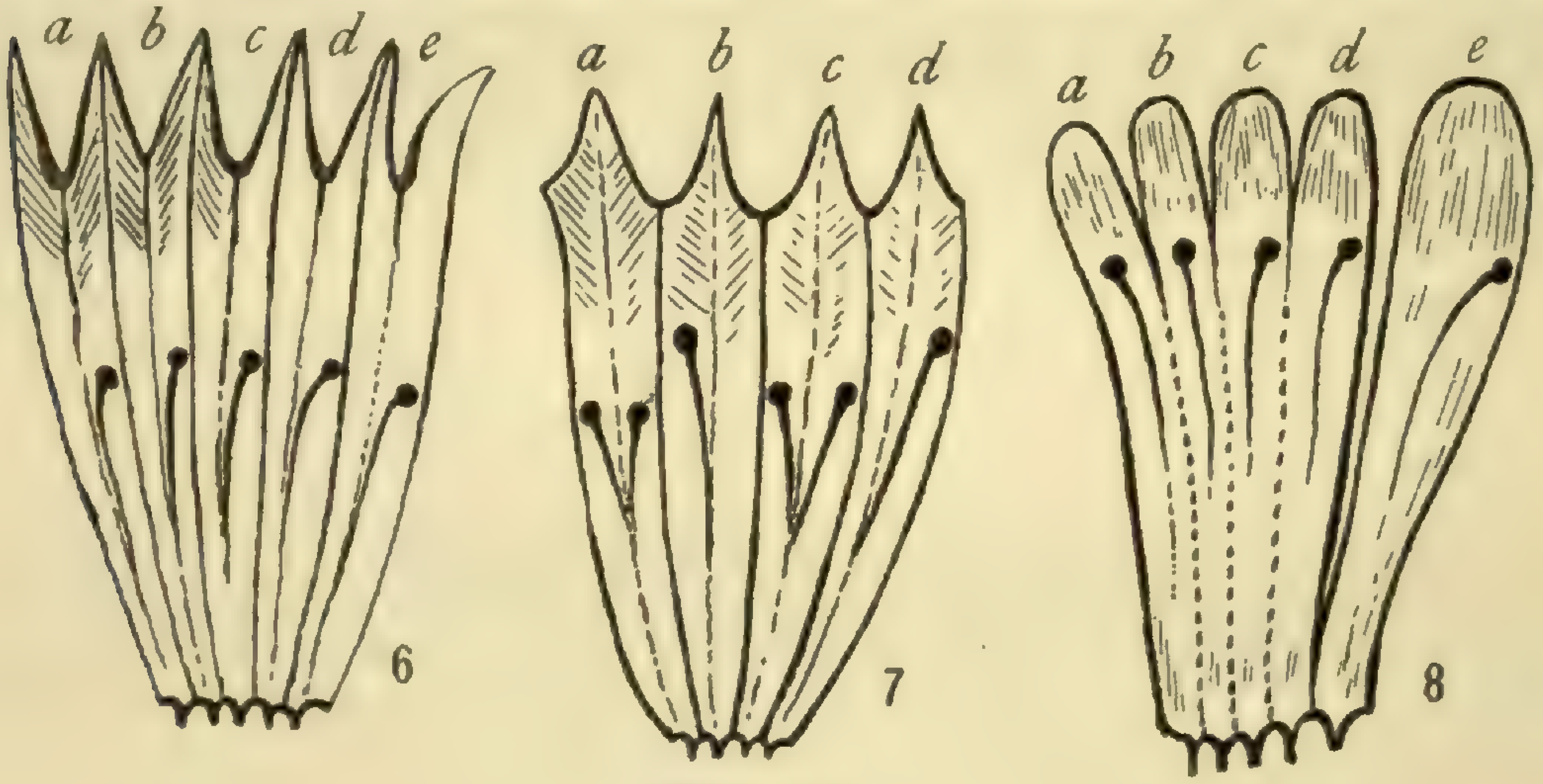
Evidently in *Agave americana* the last stage of fusion resulted in the suppression of the basal or megaspore region of three alternate fronds. Nor is it so difficult to conceive how, the plant finding the inclosure of the ovarian region to its advantage, an elongation of the style finally resulted, with the retention of the gametophytes and seeds. Also, the long filaments must have been very readily produced by the flower under the stress of impulses that had to do with nothing else than the ordinary phenomena of fertilization. Such, surely, are the conclusions one may reach from the macroscopic examination of flowers like Agave and perchance likewise of the rose hip.

While the manner of evolution just outlined is in reality not utterly different from that of the Magnoliaceae, the order in which the parts are segregated clearly indicates a remote separation of the several stocks involved, and therefore the virtual polyphyletic origin of the angiosperms. But of course, when the initial changes, and when the major or crucial changes leading up to the two groups now considered so briefly, took place, and which group is in reality the more ancient, are questions that only the future may answer.

Having seen that the ovulate region, whether locular or strobilar, and whether there are few or many ovules, offers no impassable

hiatus, it becomes desirable to interpret a few more examples of staminate organization leading toward or into the more complex types of flowers. Thus may we best test our theory and see if it applies to petals and stamens generally, and really affords a far-reaching explanation of floral constitution in the angiosperms, with ultimately a better basis of classification. But in so doing we may only take up a few forms; for an exhaustive analysis of the families based on the necessary histological and developmental data must be a truly gigantic task for many years and many workers.

Various gamopetalous flowers have the structure shown in *fig. 6*. Apparently the stamens alternate with the lobes of the corolla in



FIGS. 6–8

such forms; but on closer examination it is seen that in reality the axis of the component leaves has shortened to form the notches. This difference, so convenient in classification, therefore rests on very slight anatomical distinction, unless it can be found to accompany a particular juxtaposition of the ovules throughout the groups in question. My example is from a shrub I found growing on the slopes of Popocatepetl, though anyone may turn to Gerardia and Pentstemon as equally good examples of this type of androecium.

Again, the cruciform flower invites attention, whether it be regarded as an approach toward or a departure from distinctly gamopetalous types. For in the cruciferous flower, although the stamen is apparently inserted on the common receptacle, rather than as in the diagrammatic *fig. 7*, there is considerable doubt if it is to be regarded as of discrete origin. Instead, it would seem more probable that

although the point of stamineal attachment has become strongly depressed and indeed is virtually basal now, the flower has arisen very similarly to that shown in *fig. 4*. The two single stamens alternating with the two pairs thus form an opposing analogy to the suppression of megaspore regions in the Agave. But other explanations are also possible. For we believe that in the angiosperms petals have developed from bracts, that they have resulted from the complete sterilization of sporophylls, and that, as explained above, they result in vast numbers of instances from the apical expansion of a sporophyll, which, though greatly reduced, may still bear either megaspores or microspores or both.[3] And furthermore, we regard the stamens of Liriodendron as final reduction stages of individual sporophylls that were once large.

Of further interest in the cruciferous flower is the fact that little or no difference in the development of the petals accompanies the staminate change. But, on the contrary, in the diagram of a honeysuckle (*fig. 8*), while the stamens are of normal number and size,

[3] There also appear to be various instances of more or less completely salver-shaped corollas, in which what are usually called the sepals are in reality fertile organs alternate with the petals, between which they press and fuse up to the beginning of the lobes of the corolla, and there bear stamens just as do the petals. Take for instance those forms with four "sepals," four petals, and eight stamens borne four on the petals and four alternately between them, but plainly scaling off with the members of the calyx. The cherry (*fig. 9* from GRAY) is perchance yet another example, but one retaining more stamens, these not being so distinctly determinate in number. (They may all be of the inner row.)

FIG. 9

Perhaps as great a difficulty as we meet anywhere in applying these hypotheses of floral structure is in the case of a form like Silene with discrete hypogynous stamens and a free multiovulate ovary with an apparently distinct axial relation, for here there are several possibilities. Naturally that first coming to mind is that of a central carpellary whorl, followed below by a staminate whorl, and then by the members of the floral envelope. But another method needs to be reckoned with, namely, that which may be briefly characterized as a completed supero-axial shortening and consolidation of the bisporangiate fronds of an apical whorl, in such manner that basal megaspore-bearing pinnules assumed a vertical position and fused to form the ovary, style, and stigma, while the more apical microspore regions of these same fronds produced the stamens, the frond tips finally forming the corolla.

the members of the corolla undergo division into a major and minor group. And plainly, from these simple phases we may no doubt ultimately pass by as simple steps and combinations to a reasonable interpretation of all the multifarious reductions, suppressions, and alterations in first one set of organs, and then another, resulting in papilionaceous, orchidaceous, and all the manifold forms of angiospermous flowers.

It is obviously not feasible adequately to show the derivation and the complex relations of sporophylls leading from the primitive to the higher types in any simple manner. Above all is this true because of the necessity of taking into account the varied possibilities and phases of monoecism and dioecism in any even approximately complete presentation. And the different rates of reduction at different periods, we are bound to assume, further complicate the problem. Indeed no one diagram and no one set of diagrams would serve to outline adequately the changes now suggested. Nevertheless, the attempt is made in *fig. 10* to give the simplest and most abbreviated expression of sporophyll change possible.

Have not the transformations really been easier than we think? Dictyozamites, the net-veined cycad, and many net-veined ferns of which some were doubtless seed-bearing, together with the forms of unknown fruit like Cissites, Vitiphyllum, and Liriodendropsis, all go to show that there is no special hiatus between the angiospermous foliage and the more primitive seed-bearing plants. A single new locality in the upper Triassic or lower Jurassic may at any time completely close the foliage gap. Again, the stem structure of the angiosperms presents no difficulty of derivation from the older types, in which free branching is already known, as well as the presence of numerous flowers undergoing great reductions and changes.

The great double funnels of the near-by Ipomoea, nearly a foot in length, show how readily fusions have gone on, and how great must have been the changes, reactions, and alterations going on for ages in all floral organs. The balance once disturbed, or a critical stage or plastic form once accomplished, infinite changes in a truly polyphyletic race set in.

Other fossil evidence for metamorphosis and reduction will be forthcoming, and speedily. But the student of fossils now realizes

FIG. 10.—Theoretical and actual stages in the development of megasporophylls and microsporophylls of existing spermatophytes from hypothetical homosporous fronds.

A, homosporous early pteridophytes (Paleozoic); *B*, heterosporous pteridophyte derived from *A* (Permian?).

B′, carpellary derivative of *B* (Permian; Cycas); *B″*, staminate derivative of *B* (Permian?).

C, reduced form of *B* and a progenitor of gamopetalous angiosperms; *D*, further reduction stage of *C*, with potency of giving rise to all the essential organs and parts of a perfect flower; D'^{-V}, the stamino-petal (*D″*), carpel, stamen, petal, and sepal successively derived from *D*.

a, true carpellary leaf; *b*, reduced carpellary leaf; *c*, pedicellate ovule or seed (Cycadeoideae).

1, true staminate frond (Williamsonia, Cycadeoidea) of type leading to gamopetalous angiosperms; 2, true reduced staminate frond; 3, true stamino-petal; 4, stamen; 3′, 3″, second method of sepalo-petalous derivation.

GROUPS AND COMPLEXES.—$B'+B''$ (I–IV) = Cordaitales, Coniferales, Ginkgoales, Cycadales; $A+C$ (V) = Cycadeoideae and many proangiosperms; $A'+C$ (VI) = Magnoliaceae, Liriodendropsis, etc.; $A'+C$ (VII) = many types of naked flowers; $C+D$ (VIII) = gamopetalous and convolvulaceous forms; $D'+D''$ = many angiosperms; $A+a$ = proangiosperms.

that the proangiospermous or hemiangiospermous plant types which will exhibit critical developmental phases are likely to be inconspicuous, and that they must be studied with extreme care. Our hypothetical archaeosolandrous type, for instance, could be very easily missed, though found in excellent preservation. It will be necessary for that fortunate collector who finds the horizon and locality with lineal members of the early angiosperm line imbedded in it, to apply to his material all the newer laboratory methods, namely: (1) the staining, imbedding, and sectioning method used by JEFFREY and HOLLICK in dealing with forms apparently more like impressions or carbonaceous material than the partly preserved structures such as they finally found; (2) the developmental method, by means of which BEECHER showed the preservation of the most delicate trilobite structures, and won such clear results; (3) the collodion method of NATHORST for recovering microscopic surface details, which are often present.

That intermediate types will be found in increasing number cannot be an idle prediction. That from time to time there will be found types definitely hypothesized, may be hopefully expected. Ten years ago I predicted the discovery of the seed ferns, now known in such number and variety. Even then it had become clear that "progressive prothallial elimination with correlated spore differentiation and alteration of the frondlike sporophytes of primitive ferns of the marattiaceous or an allied group were the basal factors in the evolution of the cycadofilicinean and cordaitean alliance." And it already seemed probable that the angiosperms could be added in this statement.

At the present time these groups seem to present more and more distinct points of contact, though in a very complex manner, apparently calling into requisition nearly every thinkable modification of the monosporangiate and bisporangiate frond, both on the same and on separate axes, and with nearly every conceivable variety of rearrangement, reduction, sterilization, and suppression. Even in the Cycadales, the better known of which form a really compact group, we have been compelled to say that the orders "do not appear to have passed through precisely the same evolutionary sequence of heterospory, bisporangiate or monosporangiate, monoecious, and finally doecious fructification."

And once more, we are compelled to hypothesize an extensive and far-reaching polyphyly for the angiosperms. But, none the less, the thought is also present that once an approach has been made along all the available lines of evidence, this seeming maze of possibilities may in the end be found vastly simpler than one can now picture. At least we are already persuaded that the day will come when the true relationships and derivation of every angiospermous family will be worked out and satisfactorily stated with mathematical precision.

El Instituto Geologico Nacional
de Mexico

SAP PRESSURE IN THE BIRCH STEM

PART I

H. E. MERWIN AND HOWARD LYON

(WITH FIVE FIGURES)

During the seasons of 1902 to 1904 sap pressure observations were made on several kinds of trees in the vicinity of Oneonta, in central New York. Birches and maples illustrate the two extreme types of sap pressure phenomena. Our observations on the maples are in accord with those of other observers, especially as set forth by JONES, EDSON, and MORSE.[1] Sap pressure in the birches has not been studied much hitherto, except as incidental to other studies.

We found that glass tubes of small bore filled with mercury made very sensitive pressure gauges, especially if the tap hole in the tree and the connecting tubes were filled with water or sap when the gauge was attached. When pressure was negative (suction), a bulb tube was sometimes arranged to allow water to flow into the tap hole, and to catch the gas which escaped. Gas in the tap hole when pressure is negative causes disturbing capillary effects. Gas in the tubes has a damping influence upon the gauge.

Characteristics of sap pressure in the birches

No sap will flow from tap holes in the stem of the birch or ooze from cut twigs till the ground has thawed considerably in the spring. It is not necessary, however, that the air temperature be continuously above the freezing point before pressure becomes high. From April 5 to April 22, 1904, we have recorded seven nights in which the temperature was below freezing; yet on April 5 a positive tension of 44.3^{cm} was observed in a yellow birch (*Betula lutea*); April 9, 87^{cm} in a black birch (*Betula lenta*); April 18, 35^{cm} in a black birch. Freezing nights were often accompanied by negative pressure which was maintained for a few hours after sunrise. The maximum pressure comes about a month after the first decided appearance of pressure. The buds by this later time have begun to unfold. There is at all

[1] JONES, C. H., EDSON, A. W., AND MORSE, W. J., The maple sap flow. Vt. Agric. Exper. Sta. Bull. 103. 1903.

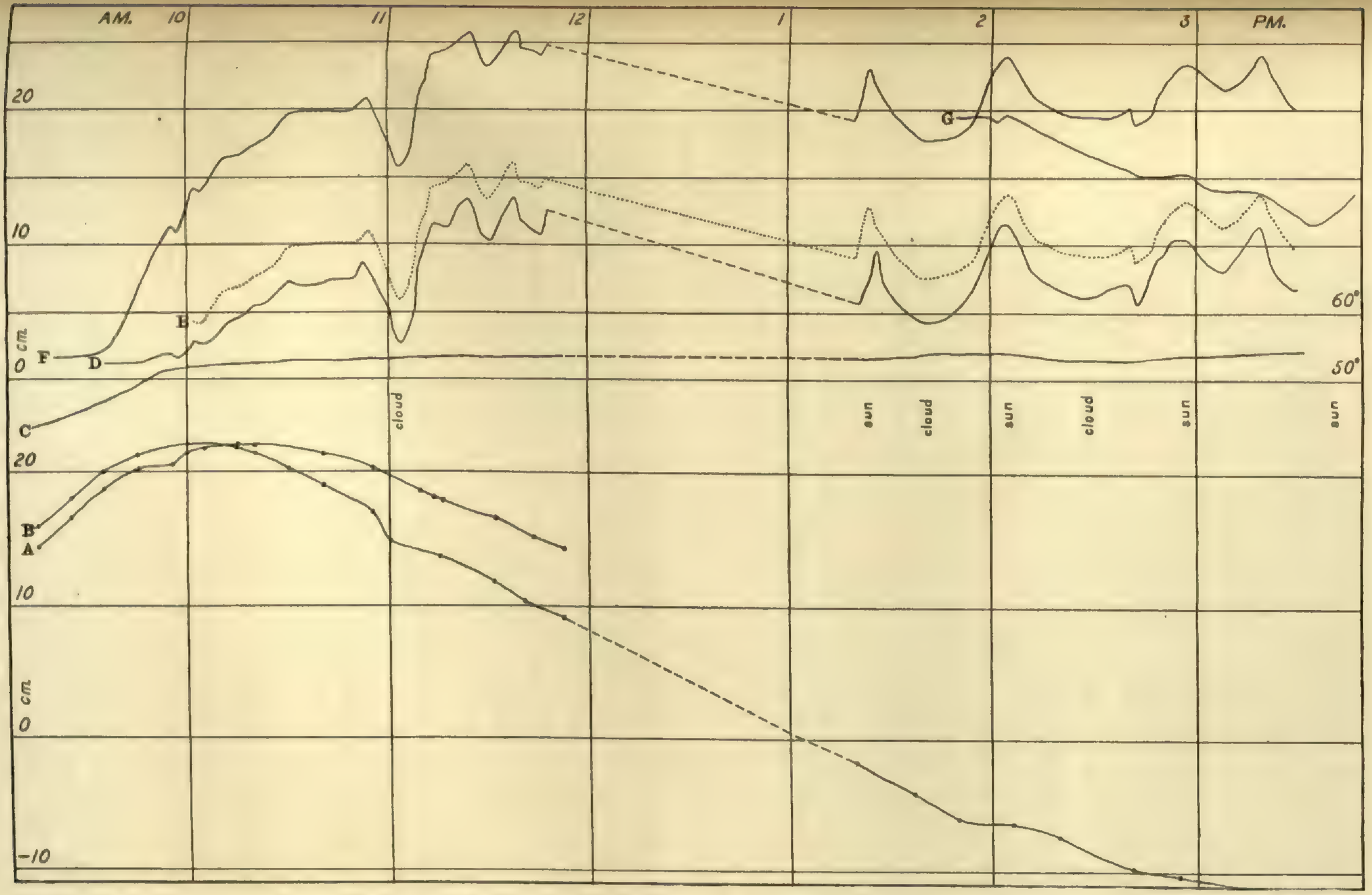


FIG. 1.—Sap pressure curves for maples and birches April 18, 1904: *A*, near the base of a 12-inch maple; *B*, 157^{cm} above *A*; *C*, the shade temperature; *D*, 180^{cm} from the base of 7-inch black birch; *F*, 135^{cm} below *D*; *E* shows where the pressure curve of *D* would have been if there had been exact hydrostatic equilibrium within the tree; *G*, curve for a 10^{cm} maple; the responses of pressure to sunshine and cloud are to be noted.

times a very regular distribution of pressure in the birch trunk, taps at the same height giving like pressures, and taps at different heights showing most pressure in the lowest. The difference between the pressure in the lowest tap hole and that in the highest is usually slightly greater than the hydrostatic difference in level between the holes (*fig. 1*).

Pressure in one hole is always immediately and markedly lowered by sap flowing freely from another hole, even though the holes are on opposite sides of the tree and many feet apart vertically. This fact, of course, indicates a free intercommunication among the ducts of the birch wood.

In all of the cases we have observed, pressure began to be evident at the base of the tree first, and as pressure increased there it showed itself higher and higher up.

Daily fluctuations of pressure in the birch were reported by CLARK.[2] The general character of these fluctuations was brought out by our observations in April 1904. A rapid rise of pressure beginning in the morning is followed by a slow decline till near sunset, then a gradual rise is kept up during the night. The nightly rise of pressure is checked if the temperature of the tree falls below freezing. Changes of pressure are only slight the next day after a freezing night, unless the air temperature reaches 40° to 45° F. or more. (These oscillations of pressure occupying a period of a day are graphically shown in *fig. 3* of the second part of this paper.)

The most striking phenomenon of the birch sap pressure is its variability during those rapid changes of sunshine that take place on days when cumulus clouds occasionally drift before the sun. We have seen the mercury column in a gauge move more than 2.5^{cm} vertically in a minute in response to a change of less than 1° C. as registered by a blackened-bulb thermometer exposed to the sun. Furthermore, pressure changes of this rapidity have been kept up for nearly ten minutes at a time. *Fig. 2* is a record for part of an afternoon in which bright sunshine and dense cloud-shadow alternated. A drop in pressure of 37.5^{cm} of mercury during a period of cloud-shadow, and a subsequent rise of 30^{cm} when the sun appeared, took place between 2:30 and 3:20 P. M. A comparison of the birch

[2] CLARK, W. S., The circulation of sap in plants. 1874.

with the maple, in respect to pressure and the passing of clouds, may be made from *fig. 1*. Even the most decided ups and downs in the curve of the birch pressure are scarcely more than suggested by the slight steepenings or flattenings in the long slopes of the maple curve.

We have always found the maximum pressures in large birches higher than in small ones. The highest pressures observed in both large and small trees were on May 2, 1904. A 7.5^{cm} black birch then gave a record of 91^{cm} (1.2 atmospheres), a 17.5^{cm} black birch of 146^{cm} (1.9 atmospheres), and a 35^{cm} black birch, about 20^{m} high, the astonishing pressure of 204^{cm} (2.68 atmospheres). The last pressure would doubtless have been even greater if it had been taken two hours earlier, for both the 7.5^{cm} and the 17.5^{cm} trees had already declined more than 10 per cent. from maximum when the large tree was tapped. As it is, this pressure is equal to 200^{cm} of mercury or 27^{m} of water, being 1.8^{m} of water higher than any previously recorded sap pressure (CLARK, *l. c.*) Such pressure would support a column of water 7.8^{m} higher than the tree. The highest point on the pressure curve of *fig. 2* represents this pressure.

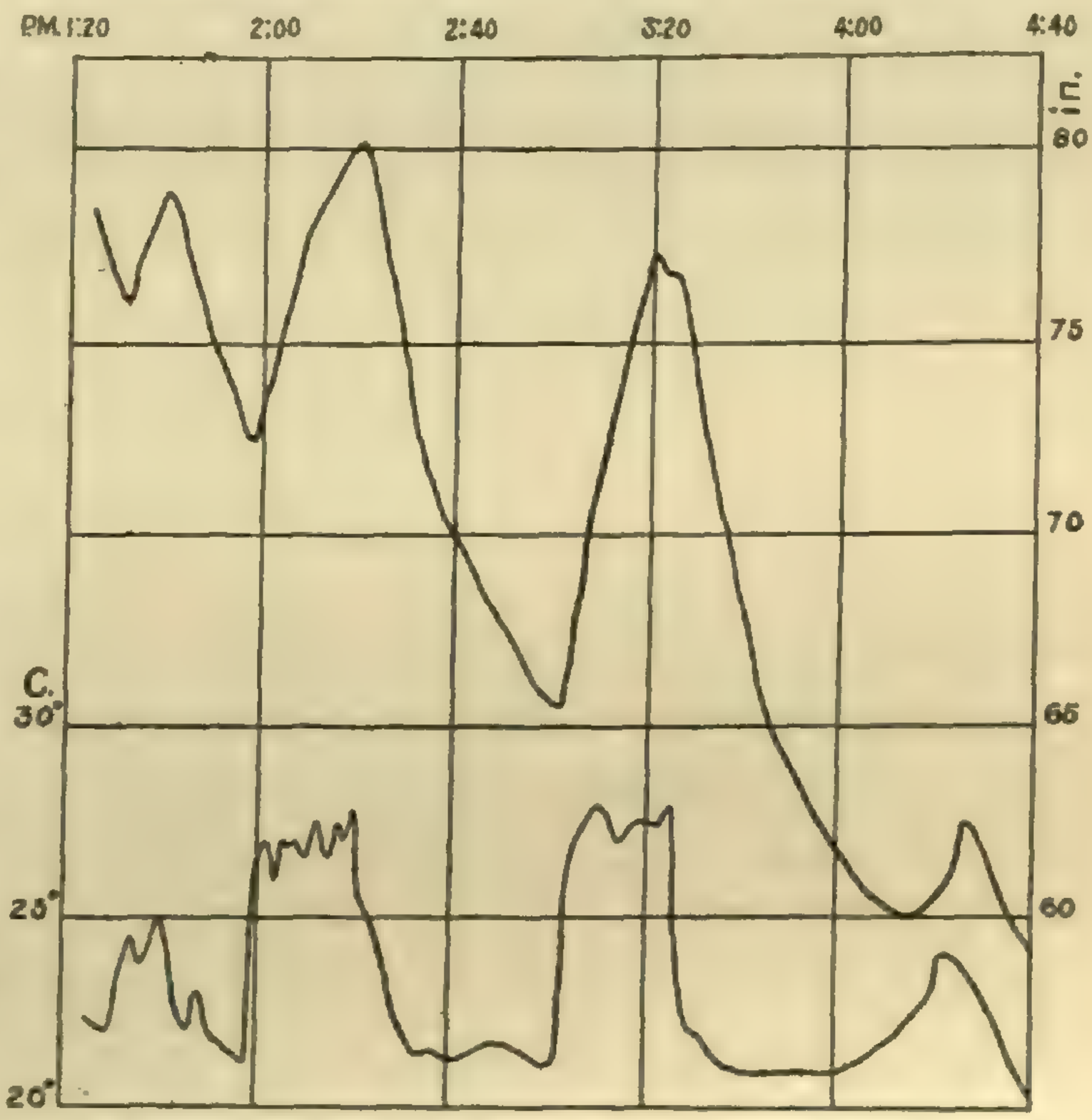


FIG. 2.—The intensity of the sunshine as measured by a blackened-bulb thermometer and the concurrent pressure, measured in inches of mercury, at the base of a 35^{cm} black birch, May 2, 1904.

Negative tensions occur frequently in the higher parts of the trunk of the birch, and less frequently near the base. In the latter position it is only in the early part of the season that suction is kept up for more than a few minutes at a time. On April 22, 1904, suction prevailed all day in yellow and black birches that had been in states of

high pressure on several occasions since April 5. A hard freeze the night before and a temperature not exceeding 40° F. during the day seem to have been the chief factors controlling the negative pressure.

The chief characteristics of sap pressure in the maple

On some warm winter days, at least as early as February 1, sap will flow in amounts of a few cubic centimeters from tap holes in small maples that are exposed to the sun; but the maximum flow and corresponding pressure do not occur till the ground is thawing in the spring. At this time pressures in a tree are not distributed with any apparent regularity. Portions of the trunk at the same level may give very different pressures, and for different heights the pressure may be greatest in either the highest or the lowest situation, though usually pressure decreases irregularly with height (*fig.* 1, *A* and *B*).

Pressure in one tap hole may be but little decreased by sap flowing freely from another hole a few inches at one side of it, but there may be a decided drop in pressure if the flow is from a hole a few feet above or below the hole to which the gauge is attached. From these facts it may be inferred that the ducts of the maple communicate with some freedom along the grain of the wood, but scarcely at all across the grain.

When pressure begins it may be manifest first either near the roots or in the branches, but for any given place in the trunk there is a strong tendency toward a daily increase of pressure during the morning hours, and a decrease during the afternoon. The decrease often goes beyond zero to a considerable suction (*fig.* 1, *B*, after 1:00 P. M.). Size of the tree, situation, and depth of tapping all affect the character of the daily pressure variation. Small size, exposure to the sun, and shallow tapping are all favorable to extreme and rapid pressure changes. In spite of all the variations already discussed, there is a tendency toward parallelism of the pressures developed in different parts of the same tree, and in various trees during a daily period. The causes of such pressure variations, as related especially to daily periods of temperature change, have been discussed by various writers.

There is a general agreement that rises and falls of temperature

of a few minutes' duration have almost no effect upon pressure in the maple. The Vermont *Bulletin* records one instance when a wavy line given by a recording gauge was probably due to variations in sunlight, pressure falling slightly when a cloud obscured the sun. Our record of April 18, 1904, shows conclusively that maples may respond notably to variations in sunlight. In *fig.* 1, lines *A*, *B*, *G* are pressure curves for maples. At 11 A. M. and at 2:00 and 3:35 P. M. the irregularities in the curves were observed to be directly related to insolation. As to the amount of tension that has been observed in maples, our highest records were from a 25^{cm} tree March 12, 1902, 75^{cm}, and from a 10^{cm} tree April 5, 1904, 69^{cm}. The Vermont *Bulletin* (p. 75) records a pressure equal to 129^{cm} on March 21, 1898. Pressures exceeding 75^{cm} are only occasionally observed. Negative pressures seldom exceed 20^{cm}.

PART II

H. E. MERWIN

Causes of sap pressure variations in the birches

The studies of 1906–1908 were carried on in Cambridge, Mass., in the hope of getting more data as to the causes of pressure variations in birches.

The character of both the long and the short period oscillations on the pressure curve, and the corresponding record of a freely exposed blackened-bulb thermometer for several days, are shown in *fig.* 3. Several important relations are to be noted between the two curves. During the day there is a close parallelism; at night the pressure curve rises regardless of temperature. In other words, maximum pressure and maximum insolation occur at about the same time, near the middle of the day; but minimum pressure comes near sunset, while minimum temperature is nearly 12 hours later, shortly before sunrise. Some of the factors in the control of sap pressure are brought out in the several experiments and discussions that follow. The details of the longer experiments referred to in the general discussion are given under a later heading.

Experiment shows that during the sap season for the birch, all the intercommunicating cavities of the roots and stem are kept practically full of sap. One tree (*exp.* 1) gave the calculated gas

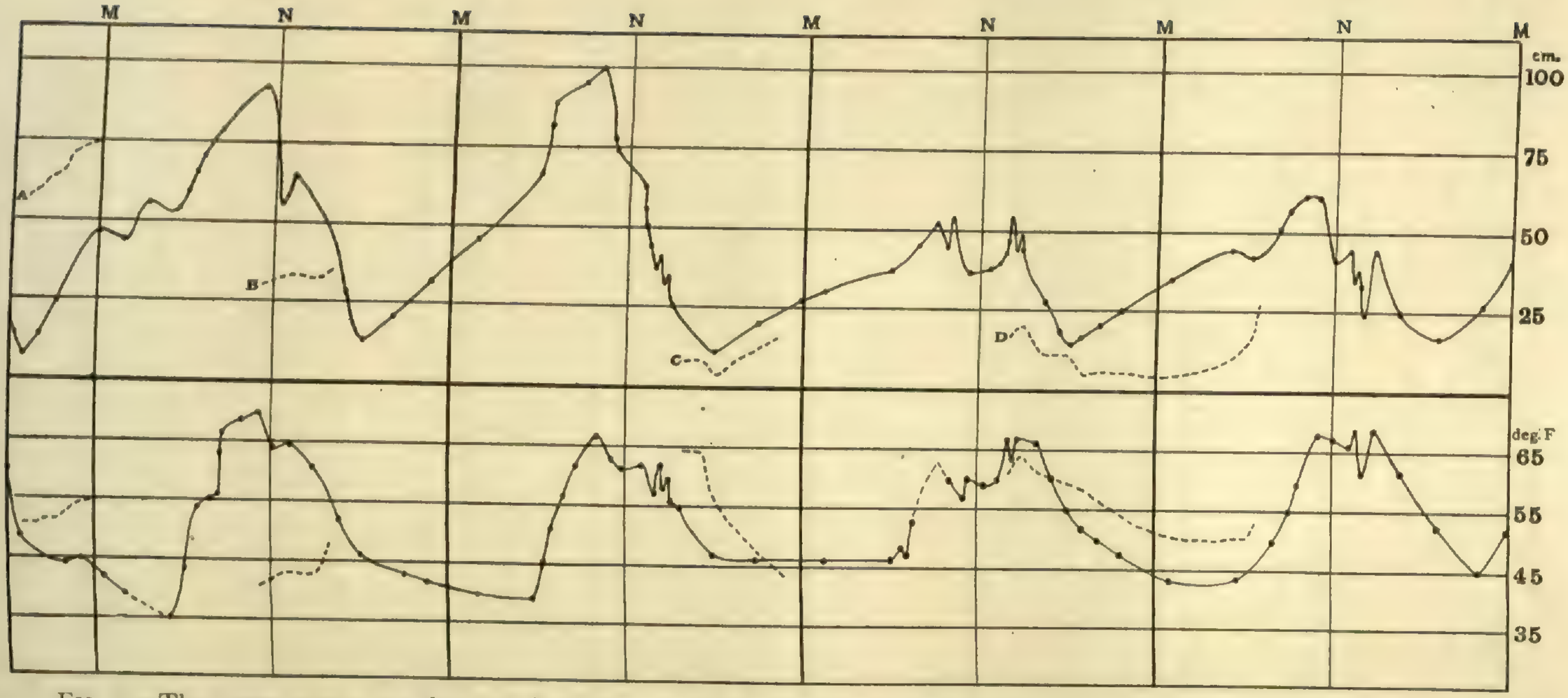


FIG. 3.—The upper curves are the records of pressure in a white birch 11cm in diameter; the continuous line covers the period April 18–21, inclusive, 1906; and the dotted lines *A*, *B*, *C*, *D* are partial records for April 14, 23, 28, 30 respectively; after April 18 the pressure was somewhat lower than it would otherwise have been, owing to a continuous slight flow of sap from the tree; the lower curves are temperatures in the sun; *M*, midnight; *N*, noon.

content of the vessels as about 1 per cent. of the total volume of the vessels. This fact easily explains the state of hydrostatic equilibrium which observation shows to exist in the birch stem when pressures are high. Increasing pressure must diminish the size of gas bubbles to a considerable extent by increasing the solubility of the gas in the sap; decreasing pressure would have an opposite effect.

There is, however, until rather late in the sap season, a good deal of gas in the closed cavities of the wood fibers. This is shown by specific gravity tests. A density of 1 is not attained in the wood of the branches until the buds are about one-third longer than in their winter condition. The maximum density of 1.14 to 1.17 is reached when the first leaves are about 8^{mm} long, near the end of the sap season. About a month is required for an increase of 25 per cent. in density. Therefore, the aerial parts of the tree considered in *exp. 1*, with an estimated volume of $57{,}000^{cc}$, would require about 500^{cc} of water as a daily supply from the roots to bring about this increase in density.

To get an idea of the amount of water required to maintain evaporation from a tree at the middle of the sap season, two twigs, weighing 1.94^{gm} and 2.3556^{gm} respectively, after the cut ends had been sealed with balsam were exposed for 3 hours, the first to a temperature of 70° F. in the laboratory, the second to about 42° F. in a breeze. The first twig lost 0.0381^{gm}, and the second 0.0256^{gm}. An average evaporation of 0.01^{gm} per hour for a twig weighing 2^{gm} may be taken, therefore, as an approximate measure of evaporation from the birch for the middle of the sap season. The tree of the experiment bore about 2000 such twigs, from which the evaporation at this rate would be 480^{cc} of water per day.

This water evaporated from the tree and that absorbed by the wood fibers during the increase of density of the tree may be taken as the approximate amount of water supplied to the tree daily by root absorption.

Inasmuch as pressures are freely transmitted throughout the birch stem, it is evident that *variations in the rate of evaporation and infiltration and of root absorption*[3] *will cause variations in pressure.*

[3] LIVINGSTON (The rôle of diffusion and osmotic pressure in plants. 1903) has discussed the factors concerned in the control of absorption and pressure in roots.

It is needless to enumerate the weather conditions which affect the rate of evaporation, but it is worth while to note at least one chief factor in the control of root absorption. It is well known that root absorption is accelerated by moderate increase of ground temperature above the freezing point. It has been shown by MACDOUGAL[4] that ground temperatures in the vicinity of New York City at a depth of 30^{cm} are maximum about 8:00 to 11:00 at night, and minimum about 8:00 to 10:00 in the morning. At greater depths the maximum and minimum would occur later, but the temperature variations would be less marked. Therefore the maximum temperature of the roots of birches—which lie mostly within less than 60^{cm} of the surface of the ground—must occur during the night, and the minimum temperature during the afternoon. Thus, root absorption and the pressure produced by it tend to increase at night. In CLARK'S (*l. c.*) experiments on roots severed from the tree, the rule was for root pressure to increase during the night and decrease during the day, for the whole period in which pressure was strong.

Taking the combined effect at night of increasing root absorption and decreased evaporation, there is a decided tendency toward an increase of pressure in the stem during the night. As the pressure increases the rate of infiltration also increases, tending thus to diminish the rate of increase of pressure (*fig. 3*). After sunrise evaporation begins to oppose the rise of pressure, so that about noon pressure begins to decline. The decreasing activity of the roots at this time aids the decline. What pressure might be developed in a birch stem by the prolonged action of root pressure, if the modifying influences of evaporation and infiltration could be eliminated, is shown by CLARK'S record of 193^{cm} pressure in a birch root severed from the stem. This pressure is more than double the pressures usually observed in the trunk.

Assuming that root pressure is essentially osmotic, the concentration of the sap in the root CLARK observed must have been about two and a half times that of the sap at the bases of trees I have observed. At different times during the sap season, I have evaporated sap from birches and found it to contain 0.5 to 1 per cent. of solids, largely

[4] MACDOUGAL, D. T., Soil temperatures and vegetation. Monthly Weather Review **31**:no. 8. 1903.

glucose. A sap containing 0.8 per cent. of glucose represents an osmotic pressure of about 78^{cm} at 0° C. It follows, then, that 100^{cm} of pressure in a birch stem is the maximum to be expected from root pressure.

Volume changes in the sap and wood due to changes of temperature in the tree cause marked variations in pressure.

I find that the expansion of sap from 6° to 32° C. is only 2 per cent. greater than the expansion of water. Cell wall substance, on the other hand, when saturated with water, expands about 2.2 times as much as water between 6° and 32° C. (*exp. 3*).

Observations as to the elasticity to the transmission of light of birch wood tissue in a thin microscopic section shows that wood fibers and the walls of the vessels in the wood have the least elasticity parallel to the length of the stem, and that the medullary rays have least elasticity along radii of the stem. In a wood fiber the greatest elasticity is perpendicular to the surface. By comparison with other substances, in which expansion by heat is directly related to elasticity to light, a different coefficient of thermal expansion for different directions would be expected in both single wood fibers and in masses of wood. My determinations made on strips of green white birch wood about 500^{cm} long immersed in water show that between 6° and 32° C. there is contraction instead of expansion in a radial direction when the temperature is raised. Under like conditions a longitudinal strip showed at first a slight expansion, but in two subsequent determinations it contracted. The coefficients of radial contraction obtained were 0.000005, 0.000004, and 0.000006; and those of longitudinal contraction were 0.000002 and 0.000003. These coefficients are so extremely small that they may be neglected in the following sap pressure calculations.[5] It thus appears that the volume changes in the cell walls above mentioned are made possible only by a diminution of the area of cross-section of the vessels and of the cavities in the cells, for the external dimensions of the tree change scarcely at all.

The effect of this tendency to diminish the pore space in the wood

[5] The thermal expansion—based upon *exp. 3* and upon the above coefficients—of a given volume of birch wood, of which 40–45 per cent. is saturated cell wall, amounts to about 1.5 times the expansion of an equal volume of water.

is to produce pressure on the liquid or gas occupying the pores. If liquid alone completely filled the cavities of the wood, any amount of thermal expansion would necessarily be accompanied by an equal amount of elastic expansion of the wood. Pressure in this case might be very great. It should be noted, however, that the pressure recorded by a gauge would be less than that developed in the tree without the attached gauge, for the sap forced from the tree into the gauge would partly relieve the pressure within the tree. It follows that the less the amount of sap required to operate a pressure gauge, the higher the pressure it will record for a given amount of thermal expansion within the tree.

It probably never happens that the wood of a birch tree becomes completely saturated with water. One or two per cent., at least, of gas is present in the wood fibers when the wood is densest. A smaller amount is present in the vessels. The compressibility of this gas lessens the effect of thermal expansion in producing pressure.

In order to obtain a quantitative statement of the amount of thermal expansion, I have made the following estimates. The small white birch (11^{cm} diameter) of *exp. 1* has about 10 per cent. of its volume in small branches and twigs. These must vary in temperature in the same way that a blackened-bulb thermometer would, only in a less degree—say a maximum daily range of 20° C. The trunk would vary less in temperature than the air—say 10° C. The maximum daily change of volume of the sap and cell walls of the tree computed on this basis would be 270^{cc}, or nearly 0.5 per cent. of the volume of the tree. Under such conditions the presence of as little as 1 per cent. of gas in the vessels would prevent an existing small pressure from rising more than about 40^{cm}. The slight increase of pressure due to the greater expansive force of the gas at the higher temperature is so small that it may be disregarded.

We may now consider in detail some of the instances in which temperature controls pressure by causing volume changes within the tree. *Fig. 2* is a record of pressure, and of temperature as given by a blackened-bulb thermometer. The periods of lower temperature were caused by the passing of clouds. Pressure increased during the periods of sunshine and diminished during the intervals of shadow. From 3:00 to 3:20, while the sun shone bright, the temperature of the

thermometer increased 7° C. The corresponding increase in pressure was 30^{cm}. It is impossible that any part of the tree except the smallest twigs could have been heated during so short a time more than 3 or 4°. There must have been, therefore, little or no gas in the vessels of the tree.

On April 21, 1906, from 2:00 to 2:45 P. M. (*fig. 3*), a rise of temperature of 4°.5 C. was accompanied by an increase of pressure of 20^{cm}.

At sunrise on each of the mornings included in *fig. 3* the pressure curves steepen greatly.

During the afternoon of April 23, 1906 (curve *B*, *fig. 3*), the pressure was rising slowly till nearly sunset in response to a change of the weather with rising temperature.

From the foregoing discussions it may be reasonably inferred that there are two chief pressure-producing agencies concerned in the phenomenon of sap pressure in the birch stem, namely root pressure and thermal expansion. The effects of both are modified considerably by evaporation and by infiltration of sap into the wood cells. Root pressure and evaporation produce a daily oscillation of pressure, with the maximum shortly after sunrise and the minimum at sunset. Thermal volume changes in the tree cause a rise of pressure from sunrise till shortly after midday, and a fall from then till sunrise. Irregular minor oscillations of short period are caused by corresponding changes in air temperature or brightness of sunshine. The combined effect of the two agencies is to make the observed maximum come about midday and the minimum at sunset. The maximum is somewhat higher than would be produced by root pressure alone—in extreme cases twice as high.

If a tree is tapped when the pressure is high, the flow of sap is at first copious, but the rate of flow lessens rapidly. The pressure, as measured anywhere in the trunk, also declines (see *exp. 2* and *fig. 5*). The relation of pressure to flow during this period of falling is different for different relative positions of the gauge and the flowing orifice. Taking a theoretical case, the pressure as distributed over a radial section of a tree before tapping is represented in *A*, *fig. 4*. Lines of equal pressure are horizontal, and pressure increases downward. Shortly after tapping at *a*, the lines of equal pressure are as shown in *B*. A little later they are as in *C*. (The diagrams are constructed

for a case in which the resistance to flow of sap is twice as great radially as longitudinally.)

Now let the rate of flow for the first ten minutes after tapping be represented by *fig. 4, D*, curve *N*, and let *B* show the distribution of pressure at the end of two minutes, and *C* at the end of the 10 minutes. Let the pressure be measured at the three points *x, y, z*. The pressure in *x* before tapping is 40.5^{cm}, at the end of 2 minutes it is 35^{cm}, and at the end of 10 minutes it is 25^{cm}. These values are plotted in *fig. 4, D*, curve *X*. The values for the pressure in holes *y* and *z* are likewise plotted in *fig. 4, D*, curves *Y* and *Z*. Inspection of these

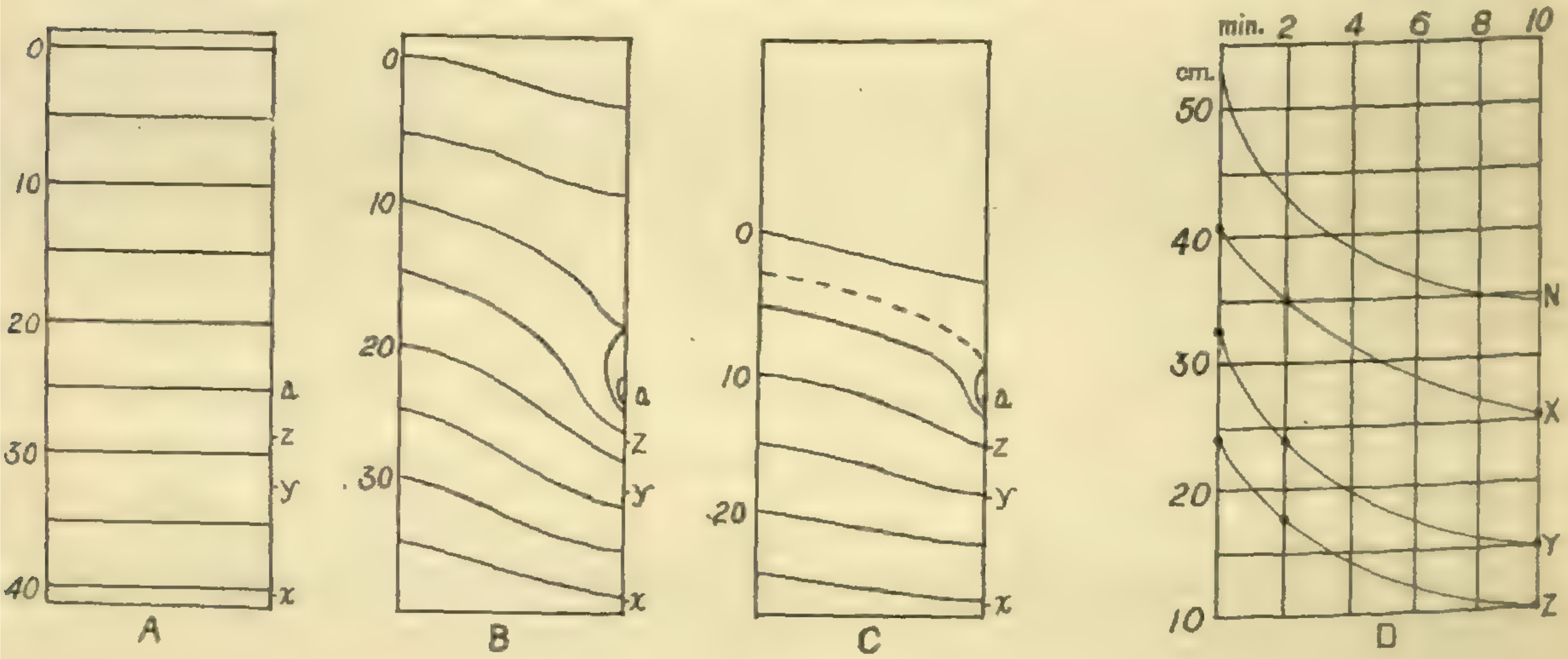


FIG. 4.—For explanation see text.

curves of pressure and the curve of flow shows that there is no definite general relation between pressure and flow.

Experiments

EXPERIMENT 1.—During the evening of April 14, 1906, while the normal evening rise of pressure was in progress, a white birch (*Betula populifolia*) 11^{cm} in diameter and 6^{m} high was tapped for two gauges 157^{cm} vertically apart. The pressure from the first was more than enough to sustain a column of water as high as the tree. The difference in pressure of the two gauges was 12 to 12.3^{cm} of mercury, the hydrostatic pressure corresponding to this difference in the height of the two gauges being 11.6^{cm}. This hydrostatic equilibrium may be explained on the supposition that a practically continuous column of water could have been traced through at least some of the ducts between the tap holes. Other ducts might have contained bubbles

of gas. That there was a small amount of gas present was shown by pouring mercury into the free arm of the lower gauge and thereby forcing back into the tree 0.45^{cc} of sap. This procedure caused the pressure within the tree to go up from 54 to 55.3^{cm}. A simple calculation shows that there was a contraction of nearly 1 per cent. in the gas in the vessels. The total volume of gas contracting was then 45^{cc}.

To get an estimate of the amount of duct space in the tree, I examined several cross-sections of branches about 2.5^{cm} in diameter. An area of $1.25^{sq\ mm}$ contained an average of 74 ducts, with an average cross-section of $0.002^{sq\ mm}$. The ducts, therefore, occupy about 12 per cent. of the volume of the tree. The volume of the tree (estimating the upper 3^{m} of the stem and branches as equal to the lower 3^{m} of the trunk) was $57{,}000^{cc}$. The duct space, then, amounted to about 6800^{cc}. Finally, 45^{cc} (the gas content of the ducts) is nearly 0.7 per cent. of the volume of the ducts. A second addition of mercury gave the gas content as 1.3 per cent. The ducts, therefore, at this time of the year were almost entirely filled with water. In a larger tree, in which there is a good deal of heart wood, there might be considerably more gas in the ducts, but many of the ducts in the heart wood are probably not freely communicating with the ducts in the sap wood.

It was noticed in connection with adding the mercury to the gauge, that after the rise of pressure thus produced had taken place, the pressure remained stationary for 15 minutes the first time, and for 30 minutes the second time, and then began rising at its previous rate. Furthermore, the length of time that pressure was stationary was such that during that time pressure would have increased naturally the same amount that it was artificially raised. We may conclude from this that the *intensity* of root pressure was increasing during the night. This is in accord with the idea that the concentration of the sap in the roots—and the corresponding osmotic pressure—becomes greater when evaporation from the branches lessens.

EXPERIMENT 2.—Before sunrise April 16, 1906, when pressure was high and slowly rising, three holes were bored in a small birch trunk, about 1.5^{m} apart vertically. Gauges were attached to the lower holes and the upper one was plugged. Equilibrium was soon established between the gauges, the upper one reading 61.9^{cm} and the

lower one 76.3^{cm}. The difference (14.4^{cm}) is 2.8^{cm} more than the pressure of the sap column between the holes. This is the condition that would obtain if sap were being artificially pumped into the base of the stem and were evaporating slowly from the top. Friction of the sap in the ducts would cause the lower gauge to read higher than if no current were flowing.

The gauge in the middle hole was then removed and the sap allowed to flow from the hole. A few minutes later the upper hole was unstopped, but no sap flowed from it, though sap had flowed from it when the hole was made. The flowing of the hole below it had caused it to cease to flow. As soon as the middle hole began flowing, the pressure in the lower hole dropped rapidly to 23.4^{cm}, and there remained nearly stationary for over an hour. The total drop in pressure in the lower hole was thus 52.9^{cm}, but in the hole above the drop was 61.9^{cm}. In other words, the pressure in the lower hole was 11.8^{cm} more than enough to raise sap to the level of the flowing hole. This, also, is a condition to be expected if a current of sap was flowing upward from the roots through the stem, overcoming friction.

The flow from the middle hole was at first rapid—17 drops in 10 seconds—but it decreased in a few minutes to 8 drops per 10 seconds, and at the end of 20 minutes to 4 drops. The flow then continued at nearly this rate for more than an hour. Curves *A* and *B*, *D* and *E*, of *fig. 5* are plotted from these observations. Curve *C* shows the drop of pressure from a similar experiment on another tree. Although the curves are nearly parallel, the ratio of flow to pressure is greater for the highest pressure than for the lowest. This relation may be explained by assuming that the copious flow of the first few minutes had a double source of supply; the larger part came from the trunk, being forced toward the tap hole by the elastic expansion of the wood and the gas in the wood; and the smaller part came as a current from the roots. As soon as the excess of pressure in the stem had been relieved, the further and nearly uniform flow was kept up by the root pressure. Changes in the degree of cloudiness produced the waviness of the curves *C*, *D*, and *E* in *fig. 5*.

During the 20 minutes that the flow was decreasing 790 drops (66^{cc}) of sap escaped. Of this not more than 40^{cc} or less than 25^{cc} could have come from the roots. (This will be seen by a study of

curve *B*.) Therefore, 32^{cc} is close to the amount supplied by the roots and therefore about 34^{cc} came from expansion within the tree. The experiment of two days before showed the amount of gas in this tree to be 45 to 80^{cc}, an amount which, by expansion, is sufficient to account for the flow here considered.

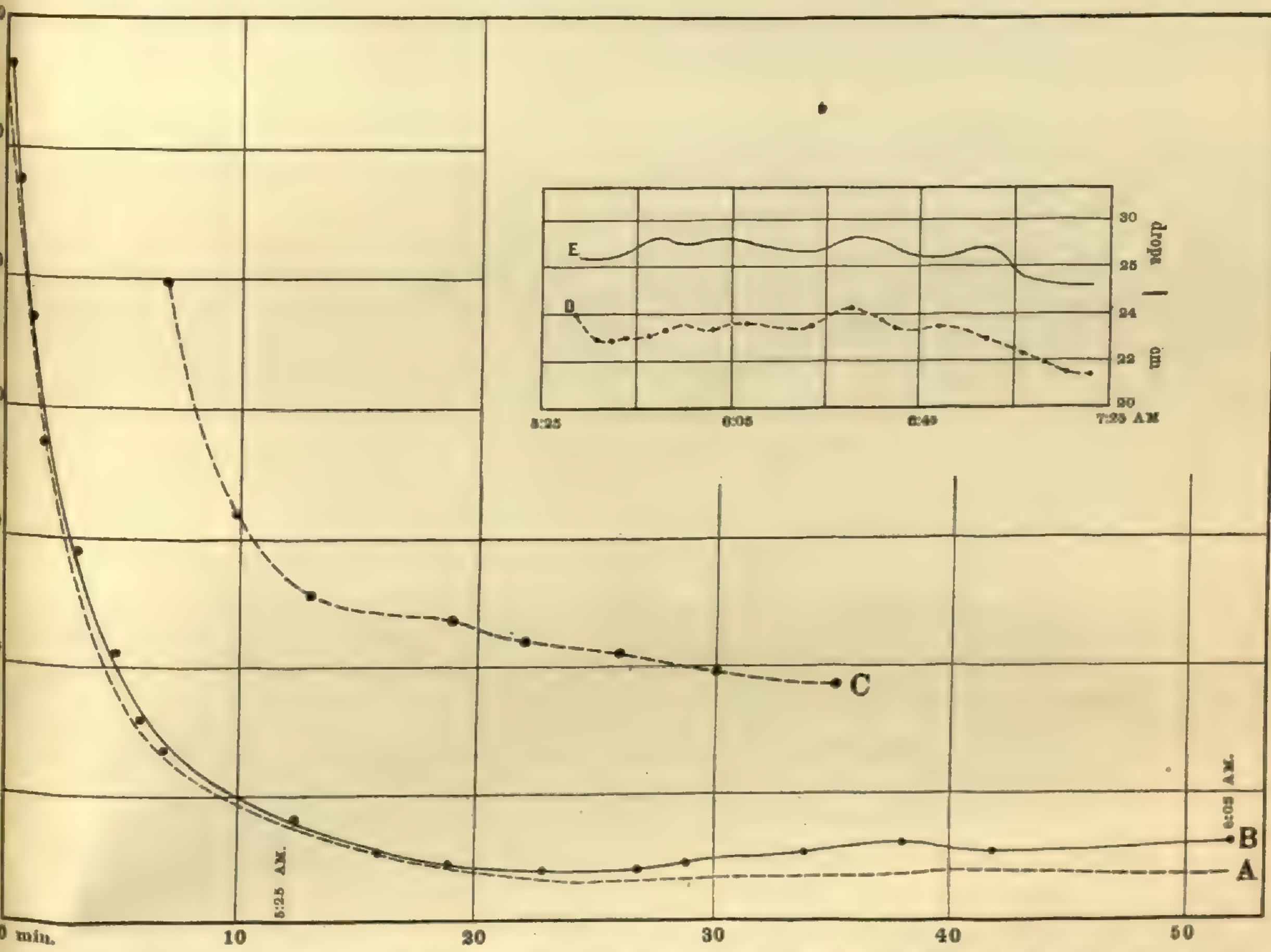


FIG. 5.—*A*, pressure at the base of a white birch, April 16, 1906, while flow was taking place from a hole higher up; *B*, rate of flow in drops per minute of the hole above *A;* the continuation of curves *A* and *B* are *D* and *E;* in another tree the decline of pressure while sap was flowing freely is shown by *C*.

EXPERIMENT 3.—To determine the amount of expansion of saturated cell-wall substance of birch wood.

Across the grain of a white birch plank thin shavings were taken. From these the air was entirely expelled under the receiver of an air pump. The shavings were then transferred to a 100^{cc} pycnometer. The pycnometer was filled up with freshly boiled, distilled water, and weighed at 6° C., and again at 32° C. The weight of the pycnometer

full of water alone was also taken at these temperatures. Finally the weight of the completely dried shavings was found. Now the specific gravity of dry cell wall substance is approximately 1.56, and the volume of saturated cell wall substance is nearly 3/2 that of dry cell wall substance.[6] Then let a = increase in weight of pycnometer full of water from 32° C. to 6° C.; b = increase in weight of pycnometer full of water and shavings from 32° C. to 6° C.; c = increase in volume of pycnometer from 32° C. to 6° C.; d = volume of pycnometer; e = volume of saturated cell wall substance; 0.0046 = expansion of 1^{cc} of water from 6° C. to 32° C. Then

$$\frac{b+c-(d-e)\left(\frac{a+c}{d}\right)}{e\times 0.0046}$$

= the ratio of expansion of cell wall to the expansion of water from 6° C. to 32° C.[7] Now, by substituting the values obtained in the experiment,

$$\frac{0.4975+0.066-(100-12.6)\left(\frac{0.433+0.066}{100}\right)}{12.6\times 0.0046}=2.2$$

= the desired ratio of expansion.

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[6] These are the figures given by SACHS, HARTIG, and others, and used in compiling the tables of the Vermont *Bulletin*.

[7] This formula would need slight corrections for more exact work, but it is more accurate than the factors of specific gravity and volume of saturated cell wall.

BRIEFER ARTICLES

CONCAVITY OF LEAVES AND ILLUMINATION

(WITH ONE FIGURE)

Concavity of the upper surfaces of leaves is of extremely common occurrence among the higher plants. WIESNER, in an important paper,[1] has discussed this concave upper leaf surface as a characteristic of the peripheral leaves of woody plants, and states that the leaves within the shadow of the crown of trees (with concave outer leaves) are generally flat or nearly so. He classes these and other leaves which are capable of some kind of temporary or permanent adjustment to the amount of light received as panphotometric leaves, regarding the concavity as useful in preventing injury to chlorophyll by excessive sunlight. OLTMANNS[2] calls attention to the fact that the leaflets of *Robinia Pseudo-Acacia* are concave on the south sides of trees, but flat on the north sides.

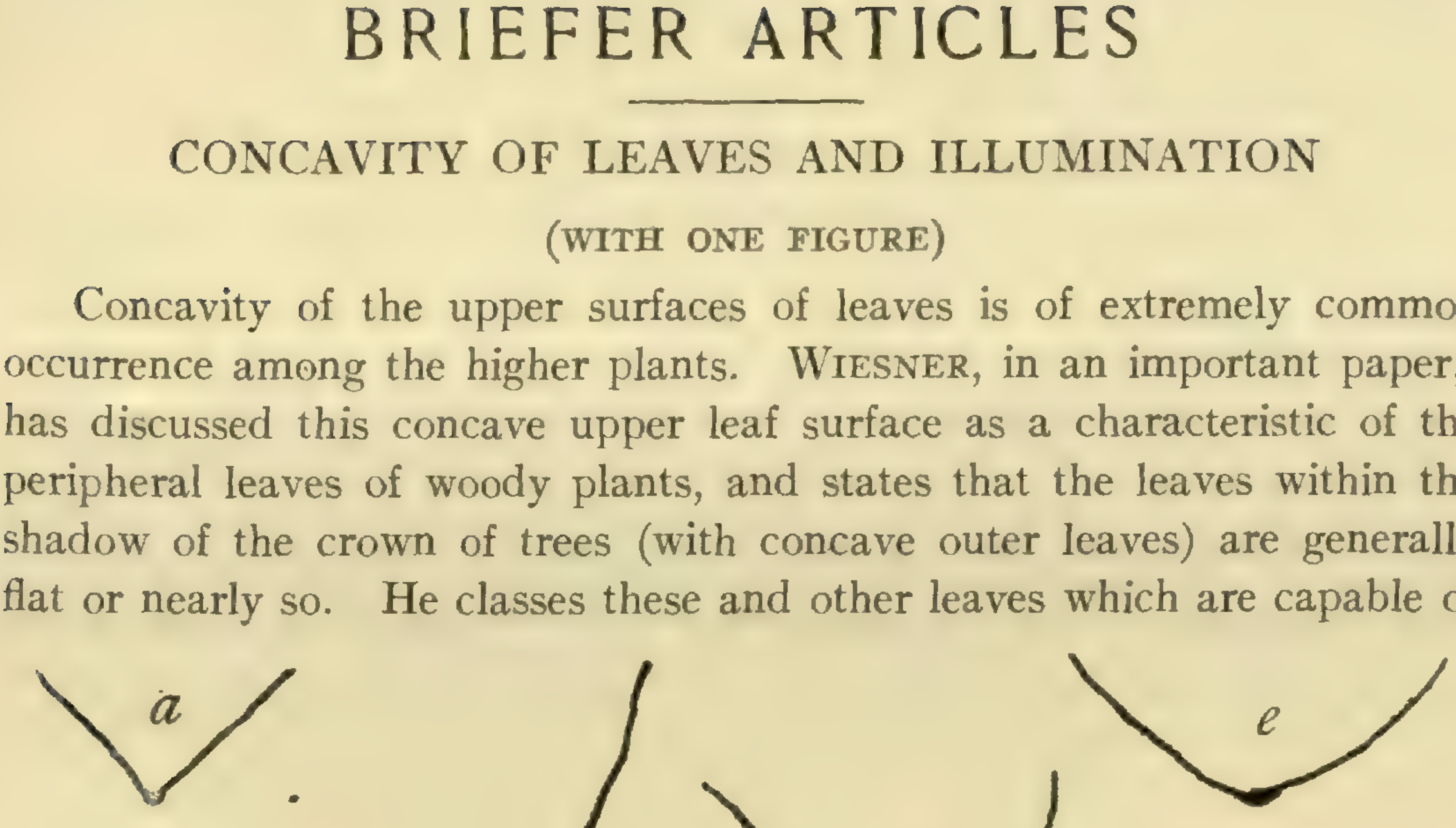

FIG. 1.—Diagram of cross-sections of leaves, taken at right angles to the midrib (if there is any) and through its middle point. $\times \frac{4}{5}$. *a*, Astilbe decandra; *b*, Prunus persica; *c*, Syringa vulgaris; *d*, Akebia quinata; *e*, Citrus medica; *f*, Styrax japonica; *g*, Begonia semperflorens; *h*, Schizophragma hydrangeoides.

It seemed to the writer worth while to make some notes as to the occurrence of the concavity in question among various genera, particularly among trees and shrubs, and to take a few measurements of the amount of con-

[1] WIESNER, J., Anpassung des Laubblattes an die Lichtstärke. Biol. Centralbl. 1–15. 1899.

[2] OLTMANNS, F., Photometrische Bewegungen der Pflanzen. Flora 79:232, 233. 1892.

cavity in shaded and unshaded leaves of the same individual. Most of the species observed were growing in the Botanic Garden of Harvard University. No attempt was made to pick out particular families for examination, but notes were made on any leaves, especially of dicotyledonous trees or shrubs, that showed decided concavity of the upper surface. Some slight amount of deviation from flatness seemed almost universal. The observations were made on mature vigorous leaves during the first week in September.

The leaves or leaflets examined in many instances showed a rather definite dihedral angle, as in *a* and *b*, *fig.* 1. More often the surfaces on each side of the midrib were decidedly curved, as in the cases *c*–*f*, while some leaves, as in *g* and *h*, showed no semblance of an angle in the cross-section, but formed a rather deep trough, with curved sides.

Measurements of angles were made by means of two straight-edged pieces of brass, hinged at one end and applied outside the leaf or leaflet at right angles to the midrib. In case the leaf surfaces were somewhat convex beneath, the angle taken was that subtended by a tangent to the middle points of the halves of the leaf.

Plants of 31 families of dicotyledons, comprising 52 genera and possibly 200 species, were noted as showing decided concavity of the upper surfaces of the leaves. Little attempt was made to find out how generally marked leaf-concavity characterized the various species of the genera examined. In some instances, e. g., Viburnum, it occurred in almost all the species accessible for observation, while in other cases, e. g., Magnolia, Acer, it was present in some species and absent in others.

The following measurements of dihedral leaf angles or concavities were obtained:

Species	Angle in degrees, sunlight	Angle in degrees, shade
Akebia quinata (leaflets)	106	167–180*
Magnolia acuminata	80–180	180 or recurved
Astilbe decandra (leaflets)	80–153	180 or recurved (167)
Hydrangea quercifolia	134–180	180 or less
Schizophragma hydrangeoides	28–180	180 or less
Fothergilla Gardeni	80–180	180 or less
Robinia Pseudo-Acacia (leaflets)	90–137	180*
Begonia semperflorens	23–56	108–180
Aralia pentaphylla (leaflets)	often semicircular	180
Ligustrum Ibota	54–145	180
Syringa vulgaris	55–145	130–180
Cephalanthus occidentalis	102–153	180
Viburnum nudum	83–105	180

* The shade leaves were all measured from the densest shade afforded by the foliage of the plant itself. In the two cases marked by an asterisk the leaves were also in the shade of other trees or a wall.

It is undoubtedly a fact that the great majority of woody dicotyledons have leaves which when freely exposed to the sun are concave on the upper surface and that this concavity usually lessens or disappears in the case of much-shaded leaves on the same plant. WIESNER'S conclusion that this conformation is due to the effects of powerful illumination seems on the whole to be plausible. It is not safe, however, to assume, as he does, that we have here an undoubted case of protective adaptation, to ward off the injurious effect upon chlorophyll of excessive insolation. In order to prove this it would be necessary to explain many real or apparent exceptions to the assumption that greater concavity should go with greater illumination. To cite the first instances that occur: *Acer Negundo* is hardly, if at all, more exposed to excessive sunlight in its usual habitats than is *A. saccharinum*, and yet the former has trough-like leaflets, while the leaves of the latter are nearly flat. The Japanese *Ligustrum Ibota* comes from a region not enough more subject to excessive sunlight than that of the European *L. vulgare* to account for the great difference in the flatness of the leaves of the two species. *Vinca rosea*, a West Indian weed, might be expected to have leaves much more concave than those of the European *V. minor*, but the reverse is actually the case. Occasionally leaves growing in shade may be decidedly more concave than those growing under greater illumination, as I have noted in one case of *Kalmia angustifolia*. Finally, it is difficult to explain on any theory of utility of concave leaf surfaces in protecting chlorophyll the fact that the angular values obtained for leaves of the same individual, grown under similar conditions, vary so much. The angles given in the preceding table for each species were in many instances obtained from leaves which apparently received almost identical illumination, since they grew fairly near together and were sometimes almost in contact with each other. Yet, as will be observed, the angles for sun leaves sometimes varied in a ratio as high as six to one. Equally difficult to explain is the fact that in some genera, e. g., Prunus, Pyrus, Salix, the whole tree may show little difference in the flatness of the leaves, all being angled whether they grow in sun or shade.

Instead of trying, as WIESNER has done, to establish the critical intensity of illumination at which leaves of a given species cease to be panphotometric and become euphotometric, it would seem to the writer better worth while to look for some relation (always on the same individual) between illumination and the average amount of angular divergence of the halves of the leaf, basing all statements on a large number of measurements.—JOSEPH Y. BERGEN, *Cambridge, Mass.*

ROEZL AND THE TYPE OF WASHINGTONIA

The palms on which WENDLAND founded his genus Washingtonia were grown from seed procured by ROEZL. They purported to have come from "Nord Mexico, bei Arizona, am Rio Colorado," but where they really came from has never been ascertained. In investigating the history of the genus it proved very difficult to obtain any account of ROEZL's American explorations. I was able to learn only of a journey made by him across the northern continent in 1872, and was therefore led to assert[3] that this was his only visit to our country. In fact, however, he had made a far more extended tour in 1868–1870, in the course of which he explored much of the United States and Mexico, and of Columbia in the southern continent. Some account of these journeyings is given in notes published by ORTGIES in volumes 20 and 23 of *Gartenflora*. Dr. TRELEASE, director of the Missouri Botanical Garden, has obligingly furnished me with an abstract of these notes, and from them I am able to present the following account of ROEZL'S explorations in the United States.

ROEZL must have gone from Europe directly to Mexico, and he spent the winter of 1868–1869 in collecting in that state and in Yucatan. In March of the latter year he sailed from Havana for New York. He then visited several of the seaboard cities and made some collections in the Allegheny mountains, after which he departed for the west by way of St. Louis, Chicago, and Omaha. On July 15, 1869, he was in Cheyenne, and on August 28 in Truckee. Considerable time was devoted to collecting in Utah and Nevada, but by November 7 he had reached San Francisco, by way of Sacramento. After a run back to Nevada City he returned to San Francisco, and went thence to San Diego. The object of his southern trip was to gather *Delphinium cardinale*, and he sent to Europe two thousand roots that he supposed to be of that species. Eventually, on flowering they proved to be one of the blue larkspurs, probably *D. Parryi*. Here also he got two plants which were introduced to European cultivation as *Yucca schidigera* and *Y. Ortgiesiana*, unquestionably the species now known as *Yucca mohavensis* and *Hesperoyucca Whipplei*.

ROEZL returned to San Francisco in time to sail on January 18, 1870, for Panama, and after making extensive collections in Columbia and Mexico, again reached San Francisco August 1, 1870.

After a week spent in Hoopa Valley, he sailed for the north, visiting

[3] BOT. GAZETTE **44**:414. 1907. Footnote 10 on this page should be corrected as follows: *dele* **1889**:330; for Jour. Bot. **1874**: read **1834**:; for Gard. Chron. **2**:521. 1889 read Gard. Chron. N. S. **24**:521. 1888.

Astoria, Portland, and Ft. Vancouver. In September he was among the mountains of the upper Columbia River, but by October he had returned to the Sierra Nevada of California. It does not appear when he finally left California, but by the middle of December 1870 he was again in Panama. The first two months of the new year were devoted to revisiting Columbia, after which ROEZL returned to Europe.

It appears from this account that the only opportunity which ROEZL had of procuring seeds of Washingtonia was during his visit to San Diego, in December 1869. The notes, however, contain no reference to this palm. But a visit to any of its desert habitats would certainly have been an experience too notable to have failed of record. Nor is it probable that his visit to San Diego, so short and so diligently occupied in collecting, could have afforded time for the difficult journey to the desert. The vague and confused habitat assigned to the palm is itself a sufficient evidence that the collector, from whom the information must have come, could never have visited a native grove. It is safe to conclude that the seed he sent to Europe came from some of the older cultivated trees at San Diego, and that his pardonable ignorance of local geography prevented him from correctly understanding what was told him of the location of the indigenous groves. —S. B. PARISH, *San Bernardino, Cal.*

LONGEVITY OF SEEDS

In the BOTANICAL GAZETTE for January 1909, p. 69, CROCKER, in referring to my paper on this subject, concludes with the remark: "I believe I am doing the author no injustice when I say that it is impossible to tell from his paper in how far it is a contribution and in how far a compilation." May I say that the utmost care was taken to quote the authority for every record or fact that was not original, and that I am unable to find a single case in which this was not done. If any such omission occurs it is a purely accidental one, and I am prepared to offer both a public and a private apology to any author whose name is omitted as the authority for a record for which he is responsible. Naturally, however, if on repeating a test or experiment a more or less divergent result is obtained, the original authority can hardly be given for the changed statement of fact, which in many cases directly negatives the original record. The latter, however, is given in all cases with the author's name appended, so that it is difficult to see any foundation for CROCKER'S criticism.—ALFRED J. EWART, *University of Melbourne.*

REJOINDER

In spite of EWART'S very energetic objection to my criticism of his paper, I maintain that the criticism is entirely justifiable. To escape personal bias I have asked several persons to read passages of EWART'S article and to say who contributed the data. In every case they decided that they were EWART'S, though this is not the case.

As an example, I cite the first part of the paragraph beginning at the foot of p. 197, dealing with *Plantago major*, *P. Rugelii*, *Thlaspi arvense*, and *Avena fatua*. The data are all given in my article,[4] but one would not know from EWART'S statement that this was their source. Again, in the paragraph beginning near the top of p. 192, EWART gives the arguments against FISCHER'S conception of the cause of delayed germination in the seeds of water plants as if based on his own work. All these arguments are given in another paper of mine,[5] issued five months before EWART'S. In the same paragraph, he says: "Since the above was written, CROCKER (BOT. GAZETTE 1907, 374) has shown, etc." One would have expected a writer who is so careful to give credit where it belongs to recast this paragraph after he discovered my article, so as to indicate proper priority. I mention these as two instances out of several that furnished the basis of my criticism.

EWART speaks again of his results contradicting mine in a number of cases. I must therefore point out again that the matters in dispute are minor details and not cardinal principles in the physiology of delayed germination; and I should be glad to have anyone compare his paper and my criticism to judge in how far there is evidence that his results disprove mine. I have repeated the experiments upon the disputed points, and have had various competent students do so independently. The results in every case agree with my previous conclusions, as my criticism points out.

I am sorry that what I considered and still consider a fair criticism has led to undue publicity. I am sure, however, that neither a public nor a private apology is necessary from EWART, as the case will rest upon its merits.—WILLIAM CROCKER, *The University of Chicago.*

[4] CROCKER, W., Rôle of seed coats in delayed germination. BOT. GAZETTE **42**: 282–284. 1906.

[5] ———, Germination of seeds of water plants. BOT. GAZETTE **44**: 375–380. 1907.

CURRENT LITERATURE

MINOR NOTICES

Malayan ferns.—This work[1] presents in a single volume a synoptical treatment of all ferns known to occur in the Malayan Archipelago; its elaboration has been carried on mainly at Buitenzorg, where the facilities for studying dried and living material of this group of plants are exceptionally good. A compendium of the families, tribes, and genera introduces the body of the work; and the sequence of families, the limitation of genera, and the nomenclature are for the most part in accordance with ENGLER and PRANTL'S *Natürlichen Pflanzenfamilien*. Fairly concise and clear keys precede the carefully drawn descriptions whenever the genus consists of two or more species, and the bibliography and synonomy are given in some detail; but it is unfortunate that the author has cited so few exsiccatae. The extended spacing and the numerous blank pages make the volume unnecessarily bulky. Moreover, the appendix of some 58 pages, treating principally of ferns discovered after January 1, 1907, and 11 pages of "additions, modifications, and corrections" which the author suggests "may be cut out and pasted on the places indicated" will mar materially the practical use of the work. It would seem almost as though publication might better have been delayed until such necessary additions and corrections could have been incorporated in the body of the text. On the whole, however, the work represents careful collation, combined with a large amount of original research, and presents in available and reliable form a comprehensive treatment of approximately 1500 species and numerous varieties, representing 10 families and 95 genera. The book doubtless will find a useful place in the taxonomic literature of ferns.—J. M. GREENMAN.

Warming's *Ecology of plants*. A correction.—My attention has been called to an unfortunate error in my review of WARMING'S *Ecology*,[2] viz., the statement that the work was written in English by the author, assisted by Dr. VAHL. As a matter of fact the work, as it has appeared, is a translation by Professors BALFOUR and GROOM from manuscript prepared by Professor WARMING and Dr. VAHL. My mistake was due to the statement on the title-page: "prepared for publication in English by PERCY GROOM," etc. (which might have been less vague if printed: "translated from manuscript," etc.), and by a personal knowledge of WARMING'S facility in the English language. It is certainly scant

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[1] VAN ALDERWERELT VAN ROSENBURGH, C. R. W. K., Malayan ferns. Handbook to the determination of the ferns of the Malayan Islands (incl. those of the Malay Peninsula, the Philippines, and New Guinea). Royal 8vo. pp. xl + 899 + 11. Batavia: Landsdrukkerij. 1908.

[2] BOT. GAZETTE **48**: 149–152. 1909.

enough credit to a translator to acknowledge his full share in such a work as this, a share that is most burdensome, and too little appreciated as a rule. English and American readers are certainly most grateful to Professors BALFOUR and GROOM for making accessible not only this new ecological treatise, but the other great ecological masterpiece as well, SCHIMPER'S *Plant geography*.—H. C. COWLES.

Pharmakognostischer Atlas.—KOCH[3] has followed his *Mikroskopische Analyse der Drogenpulver* with a second part for the use of apothecaries, wholesale druggists, sanitary officials, students of pharmacy, etc. In the arrangement of the text the author has followed his old scheme of different types, numerals, and indentations for greater facility in locating the various histological structures. Each drug has careful outlines on its preparation for microscopical observation and detailed descriptions of the individual tissues. Excellent plates of transverse and longitudinal sections serve to make these descriptions remarkably clear. The first *Lieferung* is devoted to cascarilla, red cinchona, and cinnamon barks. The complete work will certainly be useful in the recognition of crude drugs.—K. G. BARBER.

Methods in microscopy.—The second edition of the *Praktikum* of MÖBIUS[4] has about the same scope as the first. Directions are given for making preparations and also for some study of the preparations. Only the simplest methods are given, no attention being paid to the paraffin method or to critical methods of staining; in fact, most of the directions, in American schools, would be given orally or would be written on the blackboard for elementary classes which have no need as yet for any complicated technic. Of the 123 pages, 92 are devoted to spermatophytes, 6 to pteridophytes, 5 to bryophytes, and 20 to thallophytes. —CHARLES J. CHAMBERLAIN.

NOTES FOR STUDENTS

Genetics.—In the fourth report to the Evolution Committee of the Royal Society, BATESON, SAUNDERS, and PUNNETT[5] present an account of their further studies with poultry, sweet peas, and stocks. Valuable summaries are given of all the studies that have been made on these subjects, the most interesting feature being the further evidence of the occurrence of such ratios as 7:1:1:7 and 15:1:1:15. It is suggested that the types of gametic coupling evident in cases of this kind might explain the occurrence of certain aberrant forms which are generally looked upon as mutants. The term "spurious allelomorphism" is proposed

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3 KOCH, LUDWIG, Pharmakognostischer Atlas. I, Die Rinden. I Bd. 1 Lief. pp. 26. *pls.* 5. Leipzig: Gebrüder Borntraeger. 1909. *M* 3.50.

4 MÖBIUS, MARTIN, Botanisch-mikroskopisches Praktikum für Anfänger. Second edition. 8vo. pp. ii + 123. Berlin: Gebrüder Borntraeger. 1909. *M* 3.20.

5 BATESON, W., SAUNDERS, MISS E. R., AND PUNNETT, R. C., Experimental studies in the physiology of heredity. Reports to the Evolution Committee of the Royal Society 4: 1–40. 1908.

for the phenomenon of repulsion between two dominant factors or units, as for example when blueness and the erect standard in peas cannot be combined in the same individual, but are found to repel one another. The further work with stocks has mainly had reference to the phenomenon of doubling. The doubled stocks are always completely sterile and cannot be used for breeding purposes, so that it is not easy to determine the significance of this phenomenon. It is found that single stocks are of two kinds, one of which breeds true to the single, the other of which always throws a certain proportion of doubles when mated with its own kind, and the conclusion is drawn that this second type of single stock is perhaps a heterozygote in which the single character is dominant to the double. In stocks a difference is also found in the constitution of the pollen grains and ovules on the same plant. The presence and absence hypothesis is definitely accepted, and Miss DURHAM[6] in another paper appended to the report shows that on the basis of "presence and absence," the difficulties which CUÉNOT had found in certain mouse crosses completely disappear.

In a discussion of the "presence and absence" hypothesis the reviewer[7] has shown that the absence of a character may be dominant over its presence quite as well as the reverse. A simple experiment to illustrate this among the other salient features of Mendelian inheritance has also been described.[8]

He has also published the results[9] of his investigation into the elementary species and hybrids of *Bursa Bursa-pastoris* and *B. Heegeri*, showing that the leaf characters of four elementary forms which were found in nature behave in the typical Mendelian fashion on crossing with one another. The same relation is found to exist between *B. Bursa-pastoris* and *B. Heegeri* that exists among the several elementary components of the former species, thus giving new proof of the derivation of the latter species from the former. However, the *Heegeri* type of capsule which disappears in the first generation of the hybrids reappears in the second generation in a ratio of about 22:1. The important feature of these results for evolution is the fact that four pure-breeding elementary species of *B. Heegeri* resulted from the cross, when only one had been found before.

CORRENS[10] has studied variegation and yellow-green foliage in Mirabilis, Urtica, and Lunaria, in which he shows that the yellow-green form, which he

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[6] DURHAM, FLORENCE H., A preliminary account of the inheritance of coat-color in mice. Reports to the Evolution Committee 4:41–63. 1908.

[7] SHULL, G. H., The "presence and absence" hypothesis. Amer. Nat. **43**:410–419. 1909.

[8] ———, A simple chemical device to illustrate Mendelian inheritance. Plant World **12**:145–153. 1909.

[9] ———, ***Bursa Bursa-pastoris*** **and** ***Bursa Heegeri:*** **Biotypes and hybrids.** Carnegie Institution of Washington, Publ. 112. pp. 57. *figs.* **23.** *pls.* **4.** 1909.

[10] CORRENS, C., Vererbungsversuche mit blass (gelb) grünen und buntblättrigen Sippen bei *Mirabilis Jalapa, Urtica pilulifera*, und *Lunaria annua*. Zeitschr. Abstam. Vererb. **1**:291–329. *figs.* **2.** 1909.

calls *chlorina*, and the white-margined variety or *albo-marginata* are Mendelian in their hereditary behavior, both these forms being hypostatic to green and the *chlorina* hypostatic to *albo-marginata*. Two variegated varieties of *Mirabilis Jalapa*, on the other hand, give quite unexpected results. It appears that the offspring is more or less dependent upon the character of the particular branch upon which the seed was produced. BAUR[11] has found a similar behavior in the white variegated Pelargonium; thus the offspring of a pure white branch gives nothing but pure white seedlings which are incapable of successful growth, while the offspring of pure green branches produce only pure green seedlings. BAUR goes into the anatomy of the white-margined varieties, and shows that there is a complete chlorophylless sheath overlying the chlorophyll-bearing tissues, and extending beyond them at the margin. BAUR'S theory for the production of such variegated varieties is that the yellow and chlorophyll-bearing plastids are distributed into the two daughter cells at each cell division, and by chance occasionally only nonchlorophyll-bearing plastids are included in one of the daughter cells. Thereafter this white cell gives rise to a cell progeny containing no chlorophyll and thus forming a white patch or streak which is the product of this one cell. When the division is anticlinal the variegation is in blotches, and when periclinal the white tissue becomes either median or marginal, only one case of the median type having been observed. BAUR'S results seem to indicate that the male germ cells, as well as the female, contribute characteristic plastids to individuals produced by their union, but this has not yet been actually observed.

The possibility that different parts of a plant may present different hereditary qualities, as shown by CORRENS and BAUR in these variegated varieties and as very generally recognized in the case of the relatively infrequent vegetative mutations known as bud-sports, is considered a matter of considerable importance by DE LOACH,[12] who seems to have found that in certain cotton hybrids some capsules of the heterozygote have the characters of the one or the other parent practically "fixed," while others give a large degree of splitting. Such suggestions as this open up interesting fields for investigation, but theoretically it does not seem likely that different parts of the same individual plant can *generally* have its own special type of heredity.

EMERSON[13] brings together the work that has been done in the study of Mendelian characters of beans, and finds that all evidence now at hand supports the reviewer's statement that in certain varieties of beans a unit character for mottling is present, which produces an external manifestation only when in the

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[11] BAUR, E., Das Wesen und die Erblichkeitsverhältnisse der "Varietates albo-marginatae Hort." von *Pelargonium zonale*. Zeitschr. Abstam. Vererb. 1:330–351. *figs. 20*. 1909.

[2] DE LOACH, R. J. H., The Mendelian and DeVriesian laws applied to cotton breeding. Georgia Experiment Station, Bull. 83:43–63. *figs. 7*. 1908.

[3] EMERSON, R. A., Factors for mottling in beans. Annual Report American Breeders' Association **5**:368–376. 1909.

heterozygous state. EMERSON concludes that there are two distinct and unrelated units for mottling, one of which is a typical Mendelian dominant, the other becoming evident only in the heterozygous state, and on this basis he works out the expectation of various types of crosses. It remains to be demonstrated by actual breeding that such peculiar ratios may exist as 11:5, 21:38, 33:15, 16, etc.

A number of more or less popular expositions of Mendelism have appeared, and it is not necessary to mention these except in case new scientific matter has been used by way of illustration. One of the best of these general discussions is that of BAUR,[14] in which the illustrative material is almost entirely derived from his own experiments with *Antirrhinum majus*. In this species 15 or more different hereditary units have been demonstrated, and the author inclines to the view that much of so-called fluctuating variation is really Mendelian splitting of less prominent unit characters. The number of demonstrated units in this species exceeds the number of chromosomes, which leads the author to the conclusion that whole chromosomes cannot be the units upon which these characters depend. BAUR adheres strictly to the presence and absence hypothesis. SPILLMAN,[15] in a review of BAUR'S paper, shows that so far as yet demonstrated the individual chromosomes may be the fundamental bases of these unit characters, although several instances are known in which the number of demonstrated unit characters in a species is in excess of the known number of chromosomes. The crucial test must be the finding of an *individual* having more independent characters than it has chromosomes. This has not yet been done.

DARBISHIRE[16] has once again repeated one of MENDEL'S original experiments in order to determine whether there is any influence of pre-parental ancestry upon the offspring, a question involving the essential difference between the Mendelian and Galtonian conceptions of heredity. It was well that this experiment should be undertaken by one who was in the beginning a staunch Galtonian. The result of the experiment leads DARBISHIRE to the conclusion that "there is nothing like ancestral contribution within the limits of a single unit character," or in other words that segregation is perfect and the recessive character appears in as large a proportion of the offspring of a heterozygote after six generations of selection to the dominant character as in the F_2.

The Mendelian theory of heredity has produced a very deep impress upon the practical work of plant and animal breeding, and a considerable number of papers have appeared from economic sources which present valuable additional data in support of this theory, and indicating the extent of its applicability. Only a few of the more important of these papers can be noted here. Besides the paper

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[14] BAUR, E., Einige Ergebnisse der experimentellen Vererbungslehre. Beih. medizinischen Klinik **4**:265–292. 1908.

[15] SPILLMAN, W. J., The nature of "unit" characters. Amer. Nat. 43:243–248. 1909.

[16] DARBISHIRE, A. D., An experimental estimation of the theory of ancestral contributions in heredity. Proc. Roy. Soc. London B **81**:61–79. 1909.

of DE LOACH already mentioned, which shows that Mendelian behavior occurred in a certain cotton hybrid, BALLS[17] has discussed cotton hybrids in a comprehensive way, showing that at least 21 characteristics of the cotton plants behave as unit characters. BALLS declares that, while he does not know the exact number of chromosomes in the cotton nucleus, there are certainly not over 20, thus placing cotton with Pisum and Antirrhinum, as species in which the number of known unit characters exceeds the number of chromosomes. SUTTON[18] has carried on extensive crossings in Brassica and found that in a cross between the Swede turnip and the Ragged Jack kale there is a strict Mendelian behavior in which two unit characters are involved, namely, the fleshy character of the root and the curled leaves. The ratio of 9:3:3:1 appeared very clearly in the reciprocal hybrids of the cross. The glabrous-leaved brassicas (*B. oleracea* and its allies) would not cross with the hispid-leaved species (*B. rapa*, *B. campestris*, etc.), and crosses between the turnip and Swede turnip were sterile.

NILSSON-EHLE[19] maintains that practically all of the supposed mutants if not all which have been found in wheat, oats, and other similar grains split after the Mendelian fashion, and are in reality the results of occasional cross-fertilizations, i. e., of "vicinism." The most important result reported by NILSSON-EHLE is the finding of several instances in wheat and oats, in which apparently identical external characters are produced by the presence of two or more independent units, thus resulting in the F_2 ratios 15:1, 63:1, and perhaps 255:1 (observed ratio 274:1). In following generations some plants from crosses which showed 15:1 in F_2 give ratios of 3:1, and others again 15:1. In a red-grained × white-grained wheat, which presented the ratio of 63:1 in F_2, some plants in F_3 gave again the ratio of 63:1, some 15:1, and some 3:1, thus confirming the author's conclusion as to the manner in which the ratios 15:1 and 63:1 arise, and showing them to be typically Mendelian. The ligula of oats was absent in only one variety used in the experiments. This crossed with numerous other varieties gave ratios 3:1, 15:1, 63:1, and in one case apparently 255:1 (actually 274:1), in different crosses. He concludes that his results prove the correctness of the presence and absence hypothesis, and that not a single fact among his crosses is opposed to the "purity" of the gametes. He also, like BAUR, would explain the inheritance of certain apparently fluctuating characters as due to Mendelian splitting of units having similar functions.

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[17] BALLS, L., Some cytological aspects of cotton breeding. Ann. Rep. Amer. Breeders' Assoc. **5**:16–28. 1909.

[18] SUTTON, ARTHUR W., Brassica crosses. Jour. Linn. Soc. Bot. **38**:338–349. *pls. 12*. 1909.

[19] NILSSON-EHLE, H., Einige Ergebnisse von Kreuzungen bei Hafer und Weizen. Botaniska Notiser 257–294. 1908.

———, Kreuzungsuntersuchungen an Hafer und Weizen. pp. 122. 1909. Lund: Hakan Ohlssons Buchdruckerei.

EAST[20] finds that dent and flint corns differ from one another in a considerable series of correlated characters, and that these combinations of characters are maintained when these types are bred with the sugar corns. Although the flint or starch character of the grains is not then visible, breeding tests show that one or the other of these is latent, so that there is a "flint" series of sugar corns and a "starch" series.

So far as evidence goes at the present time, the Mendelian method of behavior is most clearly present in crosses between the most nearly related forms, and shows the most decided tendency to break down in the crosses between more distantly related things. For this reason all studies of the behavior of species-hybrids become of the greatest importance. HURST[21] has made a survey of orchid hybrids with special reference to the inheritance of albinism, and finds the phenomena so similar to those already worked out in sweet peas and stocks that he is convinced that they present typical Mendelian segregation. LOCK[22] gives a report on some spccies crosses of the genus Nicotiana. This is only a preliminary report and many points remain to be cleared up He finds that the second generation of some of these crosses shows a wide range of distinct types, which he believes can be analyzed on the same basis as the sweet peas and stocks have been. Color of pollen and of corolla, and form of corolla tube (bulged or funnelform) showed typical segregation. In many characters pertaining to general habit, leaf form, etc., segregation was not present or only doubtfully. LOCK suggests the possibility that "true-breeding" hybrids may result from the failure of any but homozygous combinations, this idea being made possible by the fact that very often the fertility of such hybrids is very low, so that but a small percentage of successful seeds is produced. This matter of non-splitting hybrids between species has also been presented by BURBANK,[23] who points out that this presents a method of production of new species, probably more important than usually supposed. Although several of the instances mentioned by BURBANK have not been sufficiently tested, there can be little doubt that the species-hybrids in Rubus present cases of this kind. Upon these exceptional situations the chief attention of experimenters should be concentrated.

Another hopeful direction for research that is being taken up in several quarters is that of making analyses to determine the exact nature of the unit characters. Only in this way can we hope to discover the nature of the character-producing

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[20] EAST, E. M., A note on the inheritance in sweet corn. Science N. S. **29**:465–467. 1909.

[21] HURST, C. C., Inheritance of albinism in orchids. Gardeners' Chronicle. Feb. 6, 1909.

[22] LOCK, R. H., A preliminary survey of species crosses in the genus Nicotiana from the Mendelian standpoint. Ann. Roy. Bot. Gard. Peradeniya **4**:195–227. *pls. 12.* 1909.

[23] BURBANK, L., Another mode of species-forming. Ann. Rep. Amer. Breeders' Assoc. **5**:4–41. 1909; also, Pop. Sci. Monthly 264–266. Sept. 1909.

genes. There is a growing sentiment that all hereditary phenomena must rest in last analyses upon a chemical basis. DARBISHIRE[24] has made a careful examination of the starch grains of round and wrinkled peas in pure-bred strains and in several generations of their hybrids. He finds that there are several uncorrelated features of these starch grains which result in the hybrids having some of the characteristics of the starch grains of both the parents, thus tending to obscure the fact that the segregation of the starch characters is perfect.

Miss WHELDALE[25] has studied the floral pigments of a large number of species, devoting her attention most intently to the anthocyan series, but also including a discussion of xanthein, xanthin, and carotin. A useful summary is given of the studies of others upon the chemical composition of these compounds, and a large number of observations from her own analyses are presented, the general result being to show that there is a very large number of different pigments having a common fundamental structure, which pass under each of these several names. From these analyses and from experience in cross-breeding she concludes that at least two features are necessary to the production of the anthocyan color: the one a colorless aromatic chromogen of the flavone series, the other an oxidizing agent which she believes to be a ferment. The details of Miss WHELDALE'S methods of analysis and the lengthy list of literature will prove of great value to students who desire to go into these more intimate problems of genetics.

A similar work from the animal side has been attempted by RIDDLE,[26] with respect to the melanin compounds, though the author presents no work of his own, simply bringing together in an instructive way the more recent results of investigations in this field. One unfortunate feature of RIDDLE'S work is his unfamiliarity with the Mendelian work from the experimental point of view. He thinks that he has proved the utter fallacy of the entire body of Mendelian teaching, and that he has demonstrated the continuity of the so-called unit characters, a statement which contravenes the experience of all investigators who have studied the behavior of pigment characters in actual breeding. No epigenetic hypothesis as yet gives the slightest hope of enabling such predictions as are successfully made continually by workers with the unit character conception.—GEORGE H. SHULL.

Perception of light.—HABERLANDT[27] attempts to strengthen his theory of light perception by plants, especially on the physiological side, acknowledging

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[24] DARBISHIRE, A. D., On the result of crossing round with wrinkled peas, with especial reference to their starch grains. Proc. Roy. Soc. London B **80**: 122–135. 1908.

[25] WHELDALE, MISS M., The colors and pigments of flowers, with special reference to genetics. Proc. Roy. Soc. London B **81**: 44–60. 1909.

———, On the nature of anthocyanin. Proc. Cambridge Phil. Soc. **15**: 137–168. 1909.

[26] RIDDLE, O., Our knowledge of melanin color formation and its bearing on the Mendelian description of heredity. Biol. Bull. **16**: 316–351. 1909.

[27] HABERLANDT, G., Zur Physiologie der Lichtsinnesorgane der Laubblätter. Jahrb. Wiss. Bot. **46**: 377–417. 1909.

that the experimental work there leaves much to be desired, while the anatomical relations have been pretty fully cleared up. He discusses, therefore, the investigations that have attempted to eliminate the lens action of the papillose epidermis by making plane the surface with water, oil, gelatin, etc.; after which he addresses himself to the question of the residual lens action of the epidermal cells. He shows that in all cases with oblique illumination there remains an unequal excentric distribution of the brightness on the inner walls of the epidermal cells, though of less intensity than with dry epidermis. Inquiring into the sensitiveness of the cells in distinguishing differences of illumination, it appears that the more sensitive leaves have about the same capacity, 1/30, as the human eye under ordinary circumstances, the extremes being 1/75 and 1/12.5. But the differences of illumination in the case of papillose epidermis, even when wetted, is far above these figures, and, as HABERLANDT proceeds to show, is often greater even in leaves that have the epidermal cells plane outside and concave on the inner end. In the latter case the differences were from 1/15 to 10/1, according to the obliquity of the light.

After discussing the previous more or less contradictory results of the wetting method, in which some of the experimental leaves were not in condition to perceive the direction of the light and to respond to it, he describes his new method. This consists of wetting only a part of a leaf of Tropaeolum with water made plane with a thin sheet of mica, and leaving the rest dry. A zone between the wet and dry portions is darkened with a paper screen, and the petiole is also properly protected. Then the two regions are illuminated obliquely from contrary directions. By varying the relative areas of the wet and dry regions, and the intensity of the illumination, it is easy to determine the relative efficiency of the epidermis under the two conditions. When the areas are equal and the wet area receives double the intensity of light, its direction on the dry side controls the response. When the intensities are equal and the wet area is 2.2–4.8 times the dry, the latter still dominates the reaction.

We translate the latest statement of HABERLANDT'S theory: "The perception of the direction of light results, according to my theory, from the differences in brightness or the different distribution of intensity upon the inner walls of the epidermis, which may be brought about in various ways. The smaller differences of brightnesss may be produced by the mere convexity of the inner walls of the epidermis. These *must* suffice as a stimulus (*müssen die Reizschwelle erreichen*) if the outer walls are plane and concentration of the light is thus excluded; and they *may* suffice even if the normal collection of light by convex outer walls is made impossible by wetting. Then even the wet leaf perceives the direction of the light and returns, although slowly and generally incompletely, to the usual light position. In these cases the epidermal cells act as optical stimulators, and and though they may be dispensed with to a certain extent, the promptness and precision of the movement are enhanced by their effect in increasing the intensity of the stimulus. This action is especially valuable in the last phases of the adjustment, when by the diminution of the angle of incidence, the differences of bright-

ness on the inner walls, so far as these differences are due to the convexity of the inner walls, may become less and less until they fall below the liminal value as a stimulus."—C. R. B.

Symbiosis in orchids.—A recent paper by BERNARD[28] recalls his previous studies on the germination of certain orchid seeds and on the relation of fungi to the tuberization of orchids and potatoes. The present paper advances our knowledge of these fungi and places considerable emphasis upon their place in the development of the orchid group. Three species (*Rhizoctonia repens*, *R. lanuginosa*, and *R. mucoroides*) have been recognized, described, isolated, and grown in pure cultures for considerable periods. Although no spore-bearing structures have been observed, they probably belong to the lower basidiomycetes. The first-named species seems to be the most primitive and most widespread in its symbiosis.

Two series of orchids were studied, those of epiphytic and those of terrestrial habit. The growth of the seedlings was accomplished experimentally in test tubes upon suitable media, and the effect of the fungi and of various concentrations of the media upon their development carefully studied. The results are most interesting and suggestive, and may be summarized as follows: (1) Orchids exhibit a progressive development of symbiosis corresponding to and probably in some measure the cause of their development in phylogenetic series. (2) The evolution in epiphytic and terrestrial families is parallel, and symbiosis is the only common factor which can account for this parallelism. (3) The evolution in symbiosis manifests itself in an advance from independent germination of seeds with normally developed seedlings, to a germination entirely dependent upon the infection of the embryo by fungi and the development of seedlings characterized by protocorms. In the adult plants various progressive stages of symbiosis are exhibited, from an intermittent infection with sympodial habit to permanent symbiosis associated with the monopodial habit of the host. (4) The fungi vary in virulence according to their species, their host, and the length of time they have lived outside those hosts. Very virulent cultures act upon more highly developed orchids in a similar manner to more attenuated cultures upon more primitive species. (5) Concentrated solutions of the culture media have an effect upon germination similar to that produced by infection by fungi, and both symbiosis and growth in concentrated media result in increasing the concentration of the cell contents, which seems to be the necessary condition for the development of these specialized plants.—GEO. D. FULLER.

Recent contributions from the Gray Herbarium.[29]—A. EASTWOOD (Proc. Am. Acad. **44**:563–591. 1909) has published a "Synopsis of the Mexican and

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[28] BERNARD, NOËL, L'évolution dans la symbiose. Les orchidées et leurs champignons commensaux. Ann. Sci. Nat. Bot. IX. **9**:1–196. 1909.

[29] Contributions from the Gray Herbarium of Harvard University, New Series, No. XXXVI (Proc. Am. Acad. **44**:563–637. 1909); and No. XXXVII (Proc. Bost. Soc. Nat. Hist. **34**:163–312. *pls. 23–30.* 1909).

Central American species of Castilleja" in which 54 species are recognized, 17 being new to science. A clear and concise key precedes the enumeration and description of species; the same author (*ibid.* 603–608) describes 12 new species of Mexican flowering plants belonging to different genera.—B. L. ROBINSON (*ibid.* 592–596) in a "Revision of the genus Rumfordia" records six known species, of which two are here described for the first time, and (*ibid.* 613–626) under the title "Diagnoses and transfers of tropical American phanerogams" publishes 20 new species and three new varieties, and makes several new combinations.—H. H. BARTLETT (*ibid.* 597–602) gives a "Synopsis of the American species of Litsea," recognizing 11 species, 5 of which are new, and (*ibid.* 609–612) under "Notes on Mexican and Central American alders" describes one new species and three new varieties; the same author (*ibid.*, 627–637) has published 14 new species and varieties of flowering plants chiefly from Mexico, and proposes one new genus (*Basistelma*) of the Asclepiadaceae.

J. R. JOHNSTON has recently issued, as Contribution no. 37 of the above series, a "Flora of the islands of Margarita and Coche, Venezuela," based chiefly on his own observations and collections made on the islands during two expeditions, one in 1901, the other in 1903. A brief historical sketch of the botany of the islands, an account of the physical features, a catalogue of the species, a list of the economic and medicinal plants, the distribution of species, the composition and relationship of the flora are the main topics presented, to which is added a bibliography of all works that relate directly to the vegetation of the islands. Approximately 650 species are known from Margarita and Coche at the present time; and the author estimates that this number represents about three-fourths of the entire flora. Forty-two species and two new genera have been discovered on Margarita during the course of Mr. JOHNSTON'S preparation of the present publication. The relationship of the flora, as would be expected, is with the mainland. The work forms an excellent basis for future investigations on the flora of the islands; it is, moreover, of particular scientific value since the plants on which the catalogue of species is based are deposited in several of the larger herbaria of Europe and America.—J. M. GREENMAN.

Anatomy of Zamia.—MATTE[30] has recently published an addition to the number of investigations in the interesting field of cycad anatomy. Zamia is the subject of the present work, the species studied being *Zamia floridana* and *Z. integrifolia*. The paper shows the anatomy of Zamia to be of the ordinary cycad type.

In the embryo, the vascular plate of the cotyledonary node is a protostele. Each cotyledon receives three strands, which undergo the usual branching and anastomosing, and exhibit transfusion tissue at the tips. At the base of the cotyledons the strands are mesarch and may be even concentric; they are exarch in the middle and upper regions. The first leaves are opposite, but later ones

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[30] MATTE, H., Sur la structure de l'embryon et des germinations du genre Zamia L. Bull. Soc. Sci. et Med. de l'Ouest **18**: nos. 2 and 3. 1909.

have the spiral arrangement. The leaf traces arise in the cortex, between the cotyledonary bundles, and there are three for each leaf. Girdling is acquired early. There is no clearly differentiated root structure in the embryo.

The manner of germinating is the same as that described for other cycads. The tardily appearing root is tuberized by the activity of a zone of cambium which appears immediately within the endodermis, and proliferates to such an extent that the components of the root cylinder are displaced and the cortex is exfoliated. The root cylinder may be diarch, triarch, or tetrarch, and reduces toward the tip. In its lower part all the tissues are well differentiated, even the endodermis and pericycle. The stems of young seedlings have no secondary wood, the cylinder being composed entirely of endarch leaf traces, which become mesarch farther out in the leaf. The pith of this siphonostelic stem sometimes contains a few isolated vessels. Contrary to the usual custom of looking upon these vessels as remnants of the embryonal protostele, the author prefers to regard them as vestiges of ancestral structure.

MATTE corroborates the discoveries made by LAND and the reviewer that the cotyledonary node of Zamia is of the usual cycad type, that there is a tendency to lobing at the tips of some of the cotyledons, and that there is an irregularly arranged cortical cambium, though he describes the last as vaguely cambiform, and does not attribute to it any phylogenetic significance.

The microphotographs are a retrogression from the clear and beautiful drawings of the earlier papers.—HELEN A. DORETY.

Temporary anaerobiosis.—NABOKICH has published from time to time in the past ten years short papers upon the behavior of plants under anaerobic conditions; now he gives us a monograph on the temporary anaerobiosis of higher plants.[31] There is an elaborate consideration (45 pp.) of the previous work, practically all of which is decidedly adverse to his views. Then follows the experimental part, showing how anaerobic growth is recognizable and presenting the results of an analysis of its physiological characteristics, its periodicity, dependence on temperature, rôle of sugar and alcohol, energetics, and cell division.

NABOKICH reports two categories of physiological facts which are not clearly consonant. On the one hand, anaerobic growth seems to be identical with aerobic as to the grand period, geotropic response, and cell division (including karyokinesis). On the other hand, there are peculiarities of anaerobic growth, such as the course of its curve at different periods (though this can be paralleled in aerobic growth under proper conditions), its specific dependence upon temperature and sugar solutions, and the invariable death of the cells. Though NABOKICH holds that his experiments have fully established his fundamental assumption of the capacity of higher plants for anaerobic growth, he confesses that he has not succeeded in obtaining an amount of growth beyond the limits of possible for-

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[3] NABOKICH, A. J., Temporäre Anaerobiose höherer Pflanzen. Landw. Jahrb. **38**:51–194. *pls. 1, 2. figs. 2.* 1909.

tuitous alteration in length by turgor changes; but he thinks this explanation precluded by the different behavior of the plants with sugar solutions under aerobic and anaerobic conditions respectively. Because of the great increase in growth late in the culture period, he also rejects the possibility of anaerobic growth being due to residual oxygen, even though there must be some not removable by his methods. Taking everything into consideration, NABOKICH believes that aerobic and anaerobic growth have no necessary connection, being called forth by different factors.

A reading of this paper leaves one unconvinced that the author has established his point, in spite of the apparently prodigious labor the prolonged and very difficult research has involved, with results of minimal consequence. It seems another case of the mountains in labor.—C. R. B.

Hybridization.—GIGLIO-TOS[32] publishes an attempt to reach theoretical explanation of the varied phenomena of hybridization. His views are based on his theory of "biomolecular addition" which may be briefly stated. He supposes that a fertilized egg, *A*, consists of a series of molecules *a*, *b*, *c*, *d*, *e*, ... , and that after a series of chemical transformations owing to assimilation they arrive finally at a chemical constitution *m*, *n*, *o*, *p*, *q*, ... , after which each divides into *2a*, *2b*, *2c*, *2d*, *2e*, ... , bringing a return to the original condition. When this occurs, "regeneration of the germ" would be complete. In sexual reproduction if ♂*A* nd *B*♀ represent the biomolecules of he two germ cells, the organism developed from the fertilized egg, ♂*AB*♀, might have the capacity of regenerating the whole "biomolecule" which formed it, in which case we would have parthenogenesis; or if complete regeneration is not possible we will have sexual reproduction, each of the germ cells regenerating a part. The case in plants, in which both sexes are usually present in the same individual, is not specifically considered.

From this point of view the writer interprets sexuality, synapsis and reduction, fertilization and hybridization. It is further supposed that the zygote *AB*, while retaining an equal number of male and female biomolecules, yet in the ontogeny undergoes certain modifications, so that the resulting germ cells will be ♂*M* and *N*♀; and that the constitution of these is such that they can be added to each other ("biomolecular addition") to produce again ♂*AB*♀.

On this basis the results of crossing are considered from an a-priori point of view and a number of "laws" are enunciated. Explanations of Mendelian segregation, blending, and other phenomena of hybridization are offered, and predictions made as to the results of cross-breeding reciprocal hybrids. The formal character of the hypothesis makes it probable that it will fail to conform to many of the facts of hybridism, but its viewpoint is very suggestive. For the details of its application see the original paper.—R. R. GATES.

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[32] GIGLIO-TOS, ERMANNO, L'eredità e le leggi razionali dell' ibridismo. Biologica 2:no. 10. pp. 36. 1908.

Graft hybrids.—WINKLER[33] has published a further account of his experiments with graft hybrids of *Solanum nigrum* and *S. lycopersicum*. In all, thirteen graft hybrids have appeared, belonging to five different types, which are named *S. tubingense*, *S. Darwinianum*, *S. Gaertnerianum*, *S. proteus*, and *S. Koelreuterianum*. Of these forms the first three resemble most *S. nigrum*, and the last two resemble the tomato, *S. proteus* being very variable in leaf shape and having leaves similar to *S. Darwinianum*. *S. Gaertnerianum*, like many sexual hybrids, often has sterile anthers. *S. Darwinianum* and *S. Koelreuterianum* are very unlike in their vegetative organs, but similar in their flower characters. *S. proteus* produces reversions to the tomato, which it most resembles, while *S. tubingense* reverts to the nightshade, it nearest parent.

Some viable seeds are produced by the graft hybrids, but the percentage of germination is very small. In *S. tubingense* the length of time required for ripening the fruit is short, like that of the nightshade, while the maturing time for the seeds is intermediate, and hence the ripened fruit contains immature seeds.

The chimeras described in WINKLER'S previous papers also recur, and some others are of peculiar character; e. g., one chimera was *S. lycopersicum* on one side and *S. tubingense* on the other, and another was composed of the two graft hybrid forms, *S. tubingense* and *S. proteus*. In *S. nigro-tubingense* one flower had two white petals and three yellow. *S. Darwinianum* similarly originated from a chimera which was partly *S. nigrum* and partly *S. Darwinianum*, and a pure shoot of the latter was obtained only after four decapitations of this branch. *S. Gaertnerianum* appeared five times on different grafts, in some cases as an independent shoot and in others from a chimera.

The forms are all held to be true graft hybrids and not mutations, because they are intermediate between the parents. WINKLER thinks that graft hybrids differ from sexual hybrids in their marked pleiotypy, but it is too early to say what the cause of this may be.—R. R. GATES.

Heredity in the pea.—Two papers by DARBISHIRE[34] deal with heredity in the pea. The first is a very interesting analysis of the types of starch grain in round and wrinkled hybrid peas. It is to be hoped that this valuable paper will lead to many other studies of a similar sort, because very little attention has been paid to the ontogenetic development of Mendelian characters. GREGORY[35] had previously shown that round and wrinkled peas possess different types of starch

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[33] WINKLER, HANS, Weitere Mitteilungen über Pfropfbastarde. Zeitschr. Bot. **1**:315–345. *pl. 1. figs. 4.* 1909.

[34] DARBISHIRE, A. D., On the result of crossing round with wrinkled peas, with especial reference to their starch grains. Proc. Roy. Soc. London B **80**:122–135. *figs. 6. tables 8.* 1908.

———, An experimental estimation of the theory of ancestral contributions in heredity. Proc. Roy. Soc. London B **81**:61–79. *tables 8.* 1909.

[35] GREGORY, R. P., The seed characters of *Pisum sativum*. New Phytol. **2**:226–228. 1903.

grains. In the round pea are large potato-shaped grains (*p* grains), while the wrinkled pea has compound grains (*c* grains), averaging six parts to a grain. In addition, both types possess a few very small circular grains, and in the wrinkled pea are found occasional *p* grains, though these are very rare. In the hybrid F_1 the starch grains are perfectly intermediate between those of the parents, although the character roundness is dominant. The majority of the grains in F_1 are large and round; some, however, are compound, averaging three parts to a grain. Heterozygotes (DR) in the F_5 were of a similar sort, but extracted wrinkled peas in F_5 showed an occasional *p* grain. DARBISHIRE concludes that round differ from wrinkled peas in four pairs of characters: (1) the shape of the pea, (2) its absorptive capacity for water, (3) the shape of the starch grain, and (4) the constitution of the starch grain, i. e., whether single or compound.

In a more recent paper the author tests the theory of ancestral contributions as applied to Mendelian heredity. Yellow and green peas obtained from India, Canada, China, Russia, and other sources gave similar results. The recessive character appearing in F_5 was shown to behave as though it was as pure as that borne by a pure race. It was concluded that "there is nothing like ancestral contributions within the limits of a single unit character," and that in such cases in predicting the results of a cross, "the somatic characters not only of the parents and of the ancestors of the individuals mated, but of the individuals themselves, may be left out of account," expectation being based on the theory of the contents of the germ cells.—R. R. GATES.

Diversity in cotton.—Several bulletins by COOK and his associates in the Department of Agriculture[36] are not only of great commercial value in directing the activities of cotton growers, but are also of considerable interest as studies in variability and its causes, and the results of crossing. Without attempting to mention all the topics considered, one or two of them may be referred to as of special interest. The diversity found in Egyptian cotton introduced into Arizona is considered to be of four kinds: (1) diversity due to hybridization, (2) diversity due to incomplete acclimatization, (3) diversity due directly to differences in the physical environment, and (4) diversity in different parts of the same plant. Slight differences in the external conditions have large effects in the productivity of individuals by determining the production of sterile or fertile branches.

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[36] COOK, O. F., Reappearance of a primitive character in cotton hybrids. Bureau Pl. Ind., Circ. 18. pp. 11. 1908.

———, The superiority of line breeding over narrow breeding. Bureau Pl. Ind., Bull. 146. pp. 45. 1909.

———, Suppressed and intensified characters in cotton hybrids. Bureau Pl. Ind., Bull. 147. pp. 27. 1909.

COOK, O. F., MCLACHLAN, A., AND MEADE, R. M., A study of diversity in Egyptian cotton. Bureau Pl. Ind., Bull. 156. pp. 60. *pls.* 6. 1909.

COOK. O. F., Local adjustment of cotton varieties. Bureau Pl. Ind., Bull. 159. pp. 75. 1909.

It is found that when a race of cotton is introduced into a new locality it usually shows at once an epidemic of variation in many directions, many of the plants showing a large amount of deterioration. The tendency can be eradicated only by selecting from the best (unmodified) individuals in the new locality. In this manner a reasonab y constant race is finally obtained in the new locality, the process being known as local adjustment. New-place diversity is thus a phenomenon distinct from ordinary fluctuating variability, and of prime importance in connection with acclimatization. These new-place variations are not adaptations to the conditions, but are considered to be "experiments in accommodation" or as "affording the materials from which the more definitely accommodative characters may be developed." Neither are they directly impressed upon the plants by the external conditions, but much of the diversity is believed to represent "transmitted characters which have been able to come back into expression because the change of conditions has disturbed the previous adjustments that selection had established."—R. R. GATES.

Plants with HCN.—MIRANDE finds[37] that green plants which contain cyanic compounds, if subjected to the action of chloroform, ether, and other vapors that check photosynthesis, exhale a strong odor of hydrocyanic acid. He proposes therefore to use GUIGNARD'S test[38] in connection with this process to determine what plants contain such compounds. The test requires only a short time and avoids all the complicated and troublesome processes necessary for chemical analysis. Besides it seems to be more delicate and certain. Thus MIRANDE reports that the presence of hydrocyanic acid may readily be detected in *Arum maculatum*, a plant in which the existence of HCN, long in controversy, has lately been demonstrated by analysis.—C. R. B.

Geotropism and metabolism.—The only experiments which have claimed to show a direct connection between irritability and metabolism have been those of CZAPEK and BERTEL, who found that in geotropically stimulated roots there was an accumulation of reducing substance, which they identified as homogenistic acid. The precise character of this substance has been controverted. Now come GRAFE and LINSBAUER,[39] who report that in the material used by them (*Lupinus albus* and *Vicia Faba*) the absolute amount of reducing substances (character not determined) is very small and far below the values found by CZAPEK. Moreover, there was no constant difference between the stimulated and the unstimulated roots.—C. R. B.

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[37] MIRANDE, M., Influence exercée par certaines vapeurs sur la cyanogénèse végétale. Procédé rapide pour la recherche des plantes à acide cyanhydrique. Compt. Rend. Acad. Sci. Paris **149**:140–142. 1909.

[38] BOT. GAZETTE **43**:288. 1907.

[39] GRAFE, V., AND LINSBAUER, K., Zur Kenntnis der Stoffwechseländerungen bei geotropischer Reizung. Sitzb. K. Akad. Wiss. Wien Math.-nat. Kl. **118**:907–916. 1909.

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